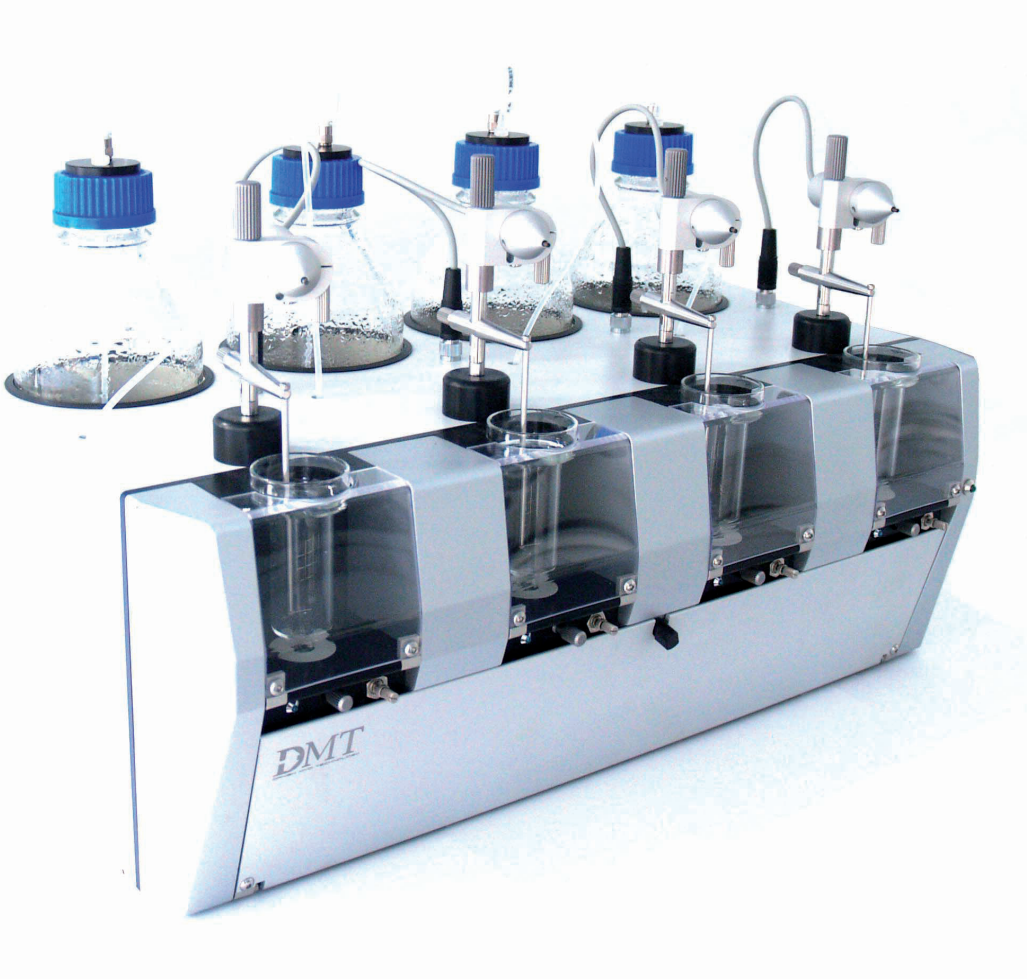
Tissue Organ Bath System

Model 750TOBS

with Data acquisition software



User Manual

Version 2.2

DMT

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Tissue Organ Bath System - Model 750TOBS

User manual

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This documentation is provided with the DMT
Tissue organ bath system – Model 750TOBS– Version 2.2

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Introduction

The vertical organ bath with its double walled glass chamber is the traditional experimental setup that has been used extensively to investigate the physiology and pharmacology of smooth muscle (vascular and non-vascular) and other tissue preparations, through measurement of contractile force while bathed in an appropriate physiological solution or buffer at 37°C. The advantages in these studies is that the preparation can be subjected to pharmacological agents while excluding the influence of systemic processes that occur in intact animals. The results of these organ bath systems are generally more consistent, reproducible and lend themselves for screening and the measurement of concentration response curves (CRCs)

The most common application of the organ bath assay are in cardiovascular research, using isolated aorta rings, heart tissue (papillary muscle) or arteries. In studying gastro-intestinal effects, preparations of ileum and colon are often used, but also gastric antral muscle and sphincter may be studied. Respiratory effects can be studied in isolated tracheal rings, phrenic diaphragm preparations, pulmonary arterial smooth muscle and even lung parenchyma. Other smooth muscle preparations that are used in organ bath research are urinary bladder, penile (corpus cavernosum) muscle strips and prostate.

Because of these inherent qualities and the versatility in use, the basic organ bath design has seen little change over the last few decades. Nonetheless, there are some practical drawbacks e.g. they take much bench space, use circulating water by causing temperature delays and gradients, are tough to clean, hard to automate, and non GLP compliant. To remedy these limitations DMT has now redesigned the organ bath system for the new millennium using the latest developments in material, computer and tissue engineering. We took away the heating by water, replaced it by very fast air heating, made the chambers disposable or reusable after cleaning, added automated control of filling, emptying and gassing by an embedded microprocessor and provide digital output of force and complete computer control of the instrument. All of this in a box that takes up a quarter of the space of a traditional system.

The modular design allows up to four individually mounted tissues to be studied simultaneously and independently per system. Moreover, multiple systems can be linked together (USB connection) for high throughput screening (HTS) and up to 16 units can easily be run by a single person. The convenient compact bench-top design of the DMT's organ bath system can be placed in any existing laboratory environment while gaining back valuable bench top space.

Safety

DMT Tissue organ bath system 750TOBS has been designed for use only in teaching and research applications. It is not intended for clinical or critical life-care use and should never be used for these purposes: nor for the prevention, diagnosis, curing, treatment, or alleviation of disease, injury, or handicap.

- Do not open the unit: the internal electronics pose a risk of electric shock.
- Do not use this apparatus near water.
- To reduce the risk of fire or electric shock, do not expose this apparatus to rain or moisture. Objects filled with open liquids should not be placed on the apparatus.
- Do not block any ventilation openings. Install in accordance with the manufacturer's instructions.
- Only use secure industry standard connectors and tubing for pressure and vacuum connections. Faults, defects and mistakes due to wrong connections void warranty. We are not accountable for results and mistakes due to inappropriate pressure or vacuum hookup.
- Do not install near any heat sources such as radiators, heat registers, stoves, or other apparatus that produce heat.
- Only use attachments and accessories specified by the manufacturer.
- Unplug this apparatus during lightning storms or when unused for long periods of time.
- This apparatus must be grounded.
- Use a three-wire grounding-type cord similar to the one supplied with the product.
- Do not defeat the safety purpose of the polarized or grounding-type plug. A polarized plug has two flat blades, one being wider than the other. A grounding type plug has two blades and a third (round) grounding pin. The wide blade or the third prong is provided for your safety. If the provided plug does not fit into your outlet, consult an electrician for replacement of the obsolete outlet.
- Be advised that different operating voltages require the use of different types of line cord and attachment plugs. Check the voltage in your area and use the correct type. See the table below:

Voltage	Line plug according to standard
110–125 V	UL817 and CSA C22.2 No. 42.
220–230 V	CEE 7 page VII, SR section 107-2-D1/IEC 83, page C4.
240 V	BS 1363 of 1984. Specification for 13A fused plugs and switched and unswitched socket outlets.

Protect the power cord from being walked on or pinched: particularly at power plugs and the point where they connect to the apparatus.
Refer all servicing to qualified service personnel. Servicing is required when the apparatus has been damaged in any way; such as, the power-supply cord or plug is damaged, liquid has spilled onto or objects have fallen into the apparatus, the apparatus has been exposed to rain or moisture, does not operate normally, or has been dropped.

EMC / EMI

This equipment has been tested and found to comply with the limits for a Class B Digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference in residential installations. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception (which can be determined by monitoring the interference while turning the equipment off and on), the user is encouraged to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different to that which the receiver is connected to.
- Consult the dealer or an experienced radio/TV technician for help.

Approvals

Complies with the EMC standards:

EMC 89/336/EEC: EN 61326-2-6:2005
EN 61000-3-2.

Certified with the safety standards:

Directive 2006/95/EC: EN 61010-1:2001
EN 61010-1/Corr.1:2003
EN 61010-1/Corr.1:2003
EN 61010-2-101:2003

Certificate of Conformity

DMT A/S, Skejbyparken 152, 8200 Aarhus N., Denmark,
hereby declares its responsibility that the following product:

DMT Tissue Organ Bath System –
Model 750TOBS, Version 2.2

is covered by this certificate and marked with CE-label conforms with the following standards:

EN 61010-1:2001 EN61010-1/Corr.1:2003 EN 61010-1/Corr.1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.
EN 61010-2-101:2003	Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
EN 61326-2-6:2005	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.
	With reference to regulations in the following directives: 2006/95/EC, 89/336/EEC

About this manual

This manual contains a complete list of procedures describing how to install, maintain and get started using the Tissue organ bath system – Model 750TOBS - Version 2.2

Chapter 1 provides an overview of the construction and basic features of the Tissue organ bath system.

Chapter 2 describes step-by-step how to setup a complete Tissue organ bath 750TOBS system including accessories.

Chapter 3 is a complete manual to the Interface. The chapter describes in detail the construction of the software and how to use all the features of the Tissue organ bath system.

Chapter 4 is a description of everyday handling of Tissue organ bath and the different kinds of mounting views available. It also contains procedures describing general as well as daily maintenance of the Tissue organ bath unit; e.g. weight calibration of the force transducers and cleaning instructions.

Chapter 5 is a list of equipment needed to setup a complete Tissue organ bath system. The chapter also contains a list of accessories and spare parts available.

Appendices contain various additional information about, Software installation, Service, Shipping instructions, Buffer recipes, System specifications and Fuse changing.

Contents

Trademarks **3**
Introduction **4**
Safety **5**
EMC / EMI **6**
Approvals **6**
Certificate of Conformity **7**
About this manual **8**
Contents **9**
Unpacking the Tissue organ bath system **11**
Chapter 1 - System Overview **12**
1.1 Tissue organ bath front panel **12**
1.2 Tissue organ bath rear panel **13**
1.3 Tissue organ bath unit **14**
Chapter 2 - Setting-up **15**
2.1 The Complete Tissue Organ bath system 750TOBS **15**
2.2 Setting up step by step **15**
Chapter 3 - User software **16**
3.1 The Software program **16**
3.2 The 750TOBS-Software User Interface **16**
3.2.1 Connect to 750TOBS: 16
3.2.2 The Main window 17
3.2.3 Drop down menus 20
3.2.3.1 File menu 20
3.2.3.2 Setup menu 21
3.2.3.3 Fill/Empty calibration 22
3.2.3.4 Pressure 22
3.2.3.5 Heating plate temperature 22
3.2.3.6 Temperature difference 23
3.2.3.7 Analog output 23
3.2.3.8 Weight calibration data 23
3.2.3.9 Wash 24
3.2.3.10 Flow calibration 24
3.2.3.11 Mode select 25
3.2.3.12 Weight calibration 28
3.3.1 View 28
3.4.1 Wash 29
3.5.1 The Dataview area: 29
3.5.2 Reading data 29
3.5.3 Comments tab 30
3.5.4 Viewing comments 31
3.6.1 Transfer data to the Datapad 32
3.6.2 Export a Datapad file to an Excel worksheet 33
3.6.3 Export a Data file to an Excel worksheet 34
3.4.2 About 35
3.4.3 Register 35

Chapter 4 - The Tissue organ bath unit 36
4.1 The first weight calibration 36
4.2 Calibration of force transducer 36
4.3 Tissue chamber handling 37
4.3.1 Adjusting the transducer 38
4.5 Tissue organ bath maintenance 40
4.6 Tissue organ bath cleaning 40
Chapter 5 - Tissue organ bath accessories and spare parts 41
5.1 General Tissue organ bath equipment 41
5.2 Tissue organ bath system accessories 42
Chapter 6 - Terms of warranty 43
Appendix 1 - Service check 44
Appendix 2 - Shipping instructions 45
Appendix 3 - System specifications 46
Appendix 4 - Fuse changing 47
Appendix 5 - Software installation 48
Appendix 6 - VCP – Driver installation 50
Appendix 7 - Buffer recipes 53
7.1 Buffer recipes 53
7.1.2 Physiological saline solution (PSS) 53
7.1.3 KPSS 55
Appendix 8 - 750TOBS controller 57
8.1 The 750TOBS-Software User Interface 57
8.1.1 Connect to 750TOBS: 57
8.2.1 The Main window 57
8.2.2 Drop down menus 58
8.2.2.1 Setup menu 58
8.2.2.2 Fill/Empty calibration 59
8.2.2.3 Pressure 59
8.2.2.4 Heating plate temperature 59
8.2.2.5 Temperature difference 60
8.2.2.6 Analog output 60
8.2.2.7 Weight calibration data 60
8.2.2.8 Wash 61
8.2.2.9 Flow calibration 61
8.2.2.10 Mode select 62
8.2.2.11 Weight calibration 64
8.3.1 View 65
8.4.1 Wash 65
8.4.2 About 66
8.4.3 Register 66

Unpacking the Tissue organ bath system

Please take a few minutes to carefully inspect your new Tissue organ bath system for damage, which may have occurred during handling and shipping. If you suspect any kind of damage, please contact us immediately and we will take care of the problems as quickly as possible. If the packing material appears damaged, please retain it until a possible claim has been settled.

We recommend that you store the packing material for any possible future transport of the Tissue organ bath system. In case of transport and the original packing material is unavailable, please contact DMT Sales Department for advice and packing instructions.

After unpacking your new Tissue organ bath system, please use the following list to check that the system is complete:

- 1 x Tissue organ bath
- 4 x Chamber units (20 ml).
- 4 x 1000ml Schott Duran Bottles incl. special designed screw caps
- 4 x Tissue holders L= 70 mm /hooks
- 1 x Tube Kit
- 1 x Calibration Kit (including marked tissue bath (20 ml) and 5 gram weight)
- 1 x USB cable
- 1 x Power cord*
- 5 meter cable air + clamps
- 2 x 7 mm fasteners
- Grease
- 1 x user manual
- PC Acquisition software

* The shape of the AC plug varies by country; be sure that the plug has the right shape for your location

Chapter 1 - System overview

1.1 Tissue organ bath front panel

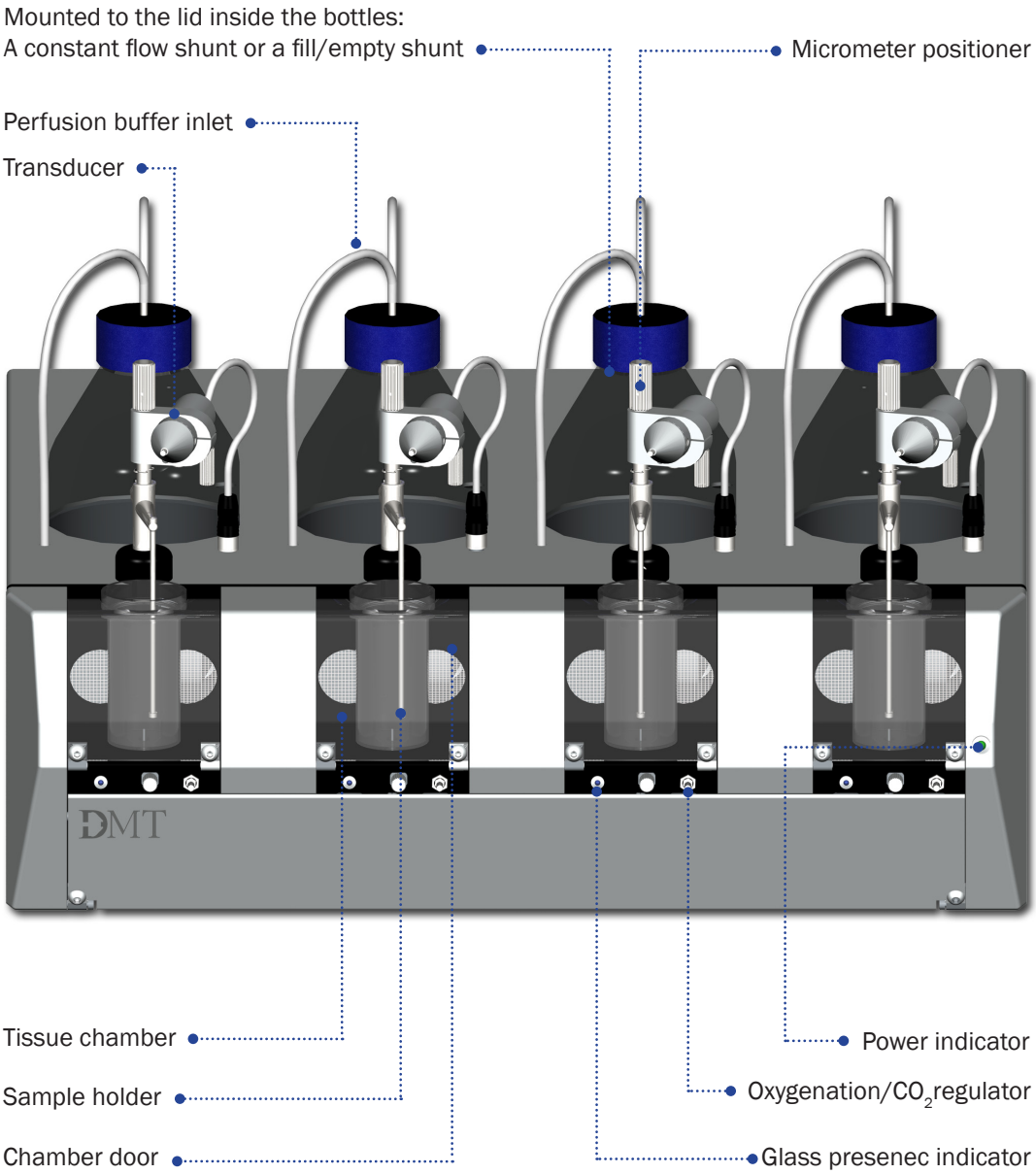


Figure 1.1 *Tissue organ bath front panel*

1.2 Tissue organ bath rear panel

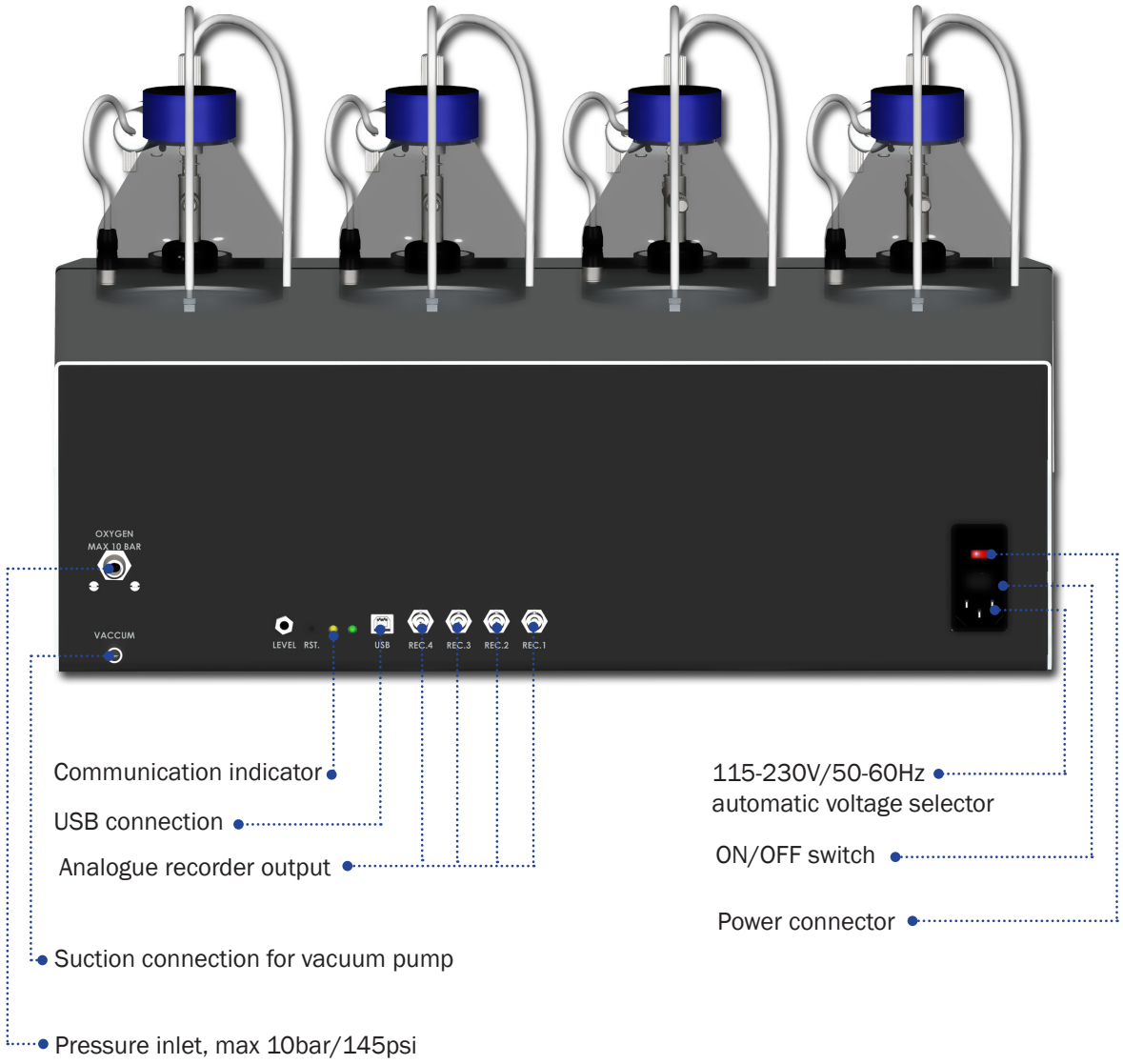


Figure 1.2 *Tissue organ bath rear panel*

1.3 Tissue organ bath unit

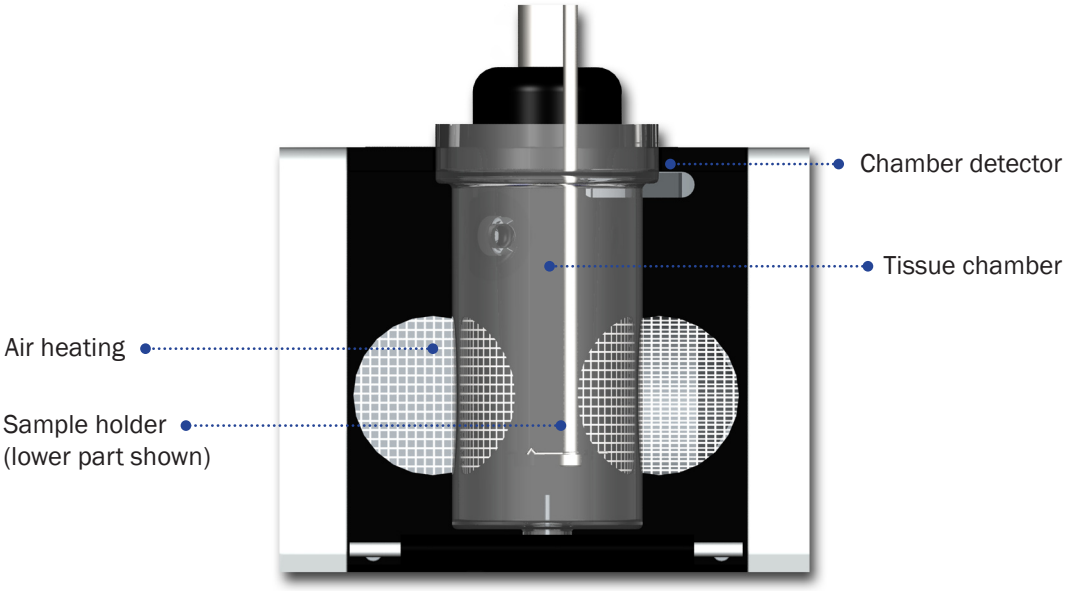


Figure 1.3 *Tissue organ bath unit*

NOTE: The chamber detector registers when the tissue chamber is placed. If the chamber is placed correctly the blue indicator lamp will be lit and the Tissue organ bath will work.

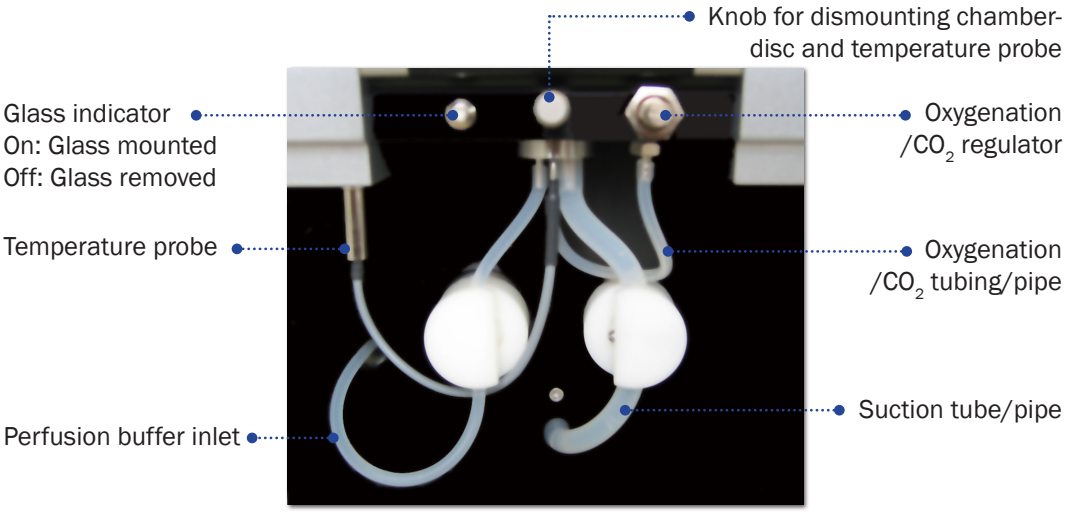


Figure 1.3a *Tissue organ bath unit*

NOTE: Regarding the Oxygenation/CO_2 regulator: The regulators need to be greased at least twice a year. Also make sure that the regulators are turned at regular intervals to prevent them from getting stuck. Use the grease that came with the system.

Chapter 2 - Setting-up

2.1 The Complete Tissue organ bath system 750TOBS

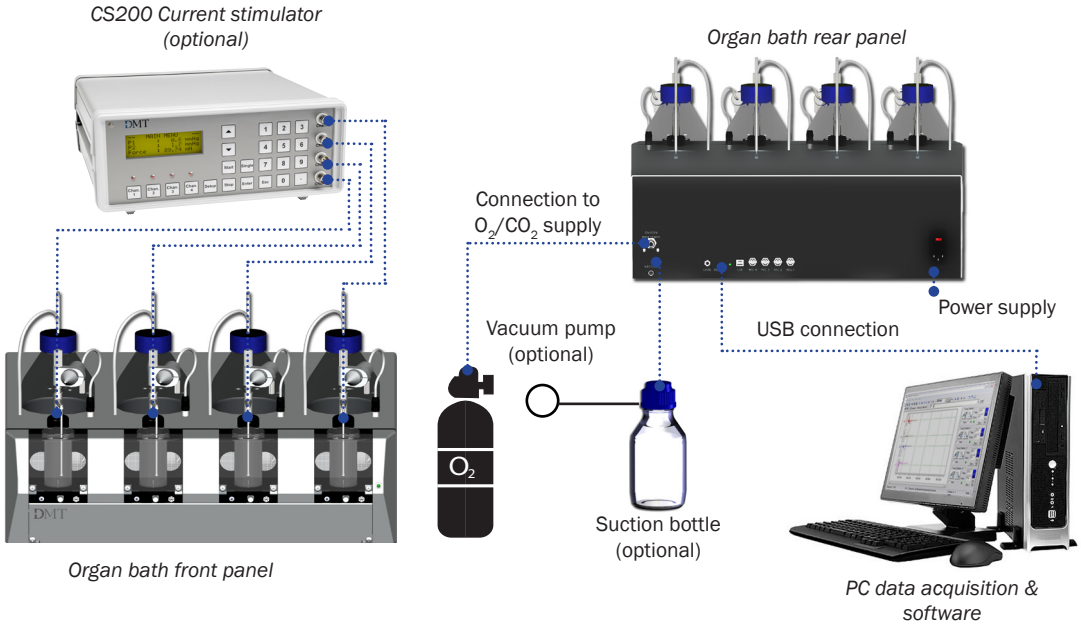


Figure 2.1 *Complete Tissue organ bath system*

2.2 Setting up step-by-step

1. **Oxygen/CO_2 supply:**
   The oxygen/CO_2 supply is attached to the pressure inlet on the back of the organ bath. After connecting the main gas supply the regulators can adjust the inlet into each chamber. Turning the regulator clockwise increases the oxygen/CO_2 supply, turning it counter clockwise decreases it.

Pressure inlet: Min. 2 bar (200 kPa, 29 PSI)
Max 8 bar (200 kPa, 116 PSI)

2. **Suction connection:**
   The system has built-in suction manifold with separate valves that allows each chamber to be drained individually. After connecting your suction device to the outlet on the back of the Tissue organ bath, all chambers or an appropriate chamber will be drained by activating the Fill & Empty Control (see 3.2.2)

When using a vacuum pump use around 11 L/min

NOTE: Please be aware of local regulations and codes for hookup to (house) vacuum systems. A VacuShield is recommended and local regulations may require shielded non-glass (Nalgene) bottles and reservoirs to be used to collect waste.

NOTE: When draining the chambers using the automatic suction function, please press the appropriate Fill & Empty control for an additional 3-5 sec. after the chamber appears to be empty. This will prevent "leftovers" from used solutions being retained in the tubing and valves.

Chapter 3 - User software

Chapter 3 is a complete manual for the Tissue organ bath Data acquisition software. The chapter contains a step-by-step description of how to navigate in the software and how to use the special features of the Tissue organ bath system. **If the user only wishes to use the 750TOBS Controller program please see Appendix 8, p. 57.**
A software installation guide can be found in Appendix 5.

3.1 The Software program

The 750TOBS-Software is generic to the Tissue organ bath application and gives the software a particular flexibility which users may find helpful in organizing their work. The software is used to control and acquire data from the 750TOBS.

The 750TOBS-Software provides many ways of displaying data:

- The grid and the colors of the dataview are changeable.
- The windows can be resized
- Expanding or contracting the X- and Y-axis can be done to meet the needs of the user
- Showing all four channels or just one channel.

There are three tabs:
1. The dataview tab, showing the data collected from the four force transducers.
2. The comments tab showing a log of all user actions done on the four chambers.
3. The Data Pad tab, showing user chosen data from the dataview.

The acquired data, comments and the Data Pad, can be exported to an Excel Worksheet for further processing.

3.2 The 750TOBS-software User Interface

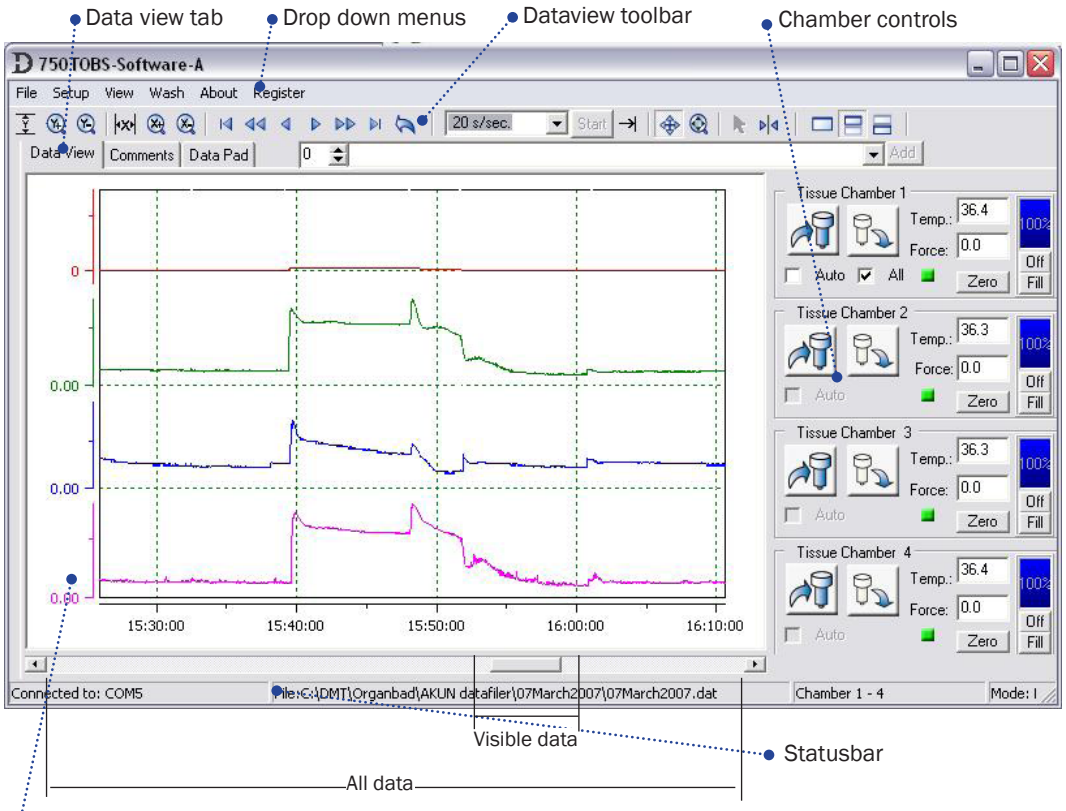
The first time the DMT Tissue organ bath is turned on, the VCP drivers must be installed as described in Appendix 6.

3.2.1 Connect to 750TOBS:

Setup->Connect to 750TOBS:
The connection to the 750TOBS is automatic. When the connection is performed, the message “Connected: COMx” is displayed in the status bar in the bottom left corner of the program window. If the connection fails, the message display is: “Not connected”.
In case the connection fails try connecting by clicking on ‘Setup->Connect’. If the connection fails again, check if the USB cable is mounted correctly and 750TOBS is turned on.

If two 750TOBS are connected to one PC use both programs. **Always connect 750TOBS-Software A before 750TOBS-Software B.**

3.2.2 The Main window



- Activate an axis by left clicking on it. The activated axis is marked by a black rectangle. The axis can now be scrolled or zoomed using the scroll or zoom tool. Arrow keys, PageUp, PageDown, Home and End buttons can also be used on an active axis.

Figure 3.1 *The Tissue organ bath User Interface*

DataView toolbar

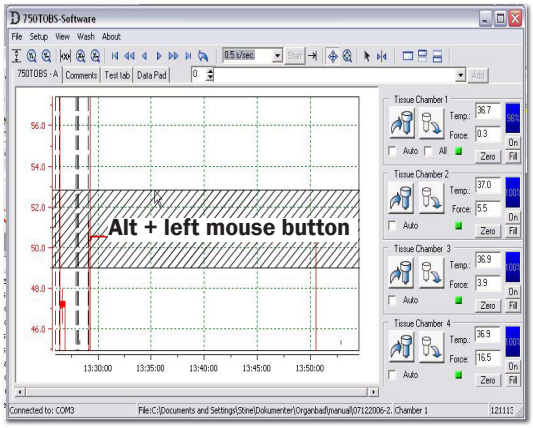
- **View all**
- **Increase X axis**
- **Decrease X axis**
- **View all**
- **Increase Y axis**
- **Decrease Y axis**

- **Go to the beginning of the file**
- **Go to the beginning, one page at a time**
- **Go to the beginning, 1/10 page at a time**
- **Go to the end of the file**
- **Go to the end, one page at a time**
- **Go to the end 1/10 page at a time**
- **Undo up to the last 10 zoom actions**
- **Start/Stop recording**
- **Choose sample rate**

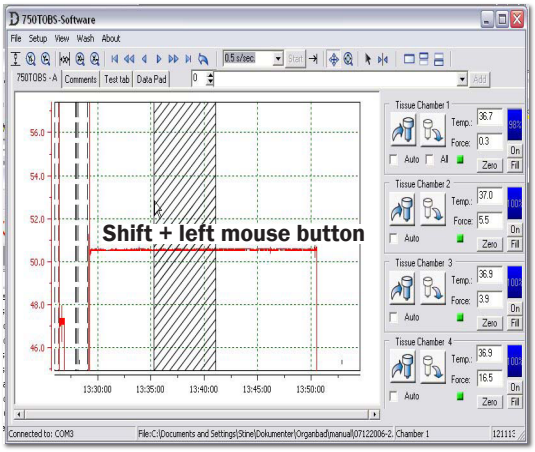
- **Resume to the end of the datasheet**
- **Scroll**
- **Zoom**
- **Select:** Activate to select comments in the dataview. To view a comment hold the cursor above a comment line, left click and a new window will open showing data from the selected comment.
- **Cursor:** The cursor is moved by holding the left mouse button while moving the mouse. There are several options for reading data. Right clicking on the cursor opens an options menu.
- **Full screen**
- **Upper half** (when 2 programs are open at the same time)
- **Lower half** (when 2 programs are open at the same time)

Zoom selection of data in the Data view:
An easy way of selecting data in the Data View is by dragging the mouse over the area while holding down the Alt key (X-axis) and the left mouse button or the shift key (Y-axis in the one channel view only) or the Alt+shift (X- and Y-axis in the one channel view only).

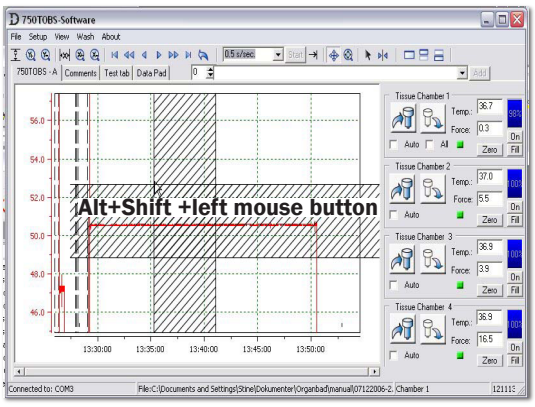
Zoom in on the X-axis:
Four and one channel view



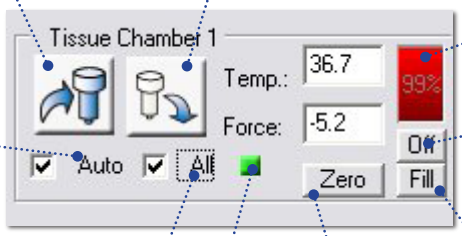
Zoom in on the Y-axis:
One channel view only



Zoom in on the X-axis and Y-axis:
One channel view only



Tissue chambers controls



Fill chamber

Empty chamber

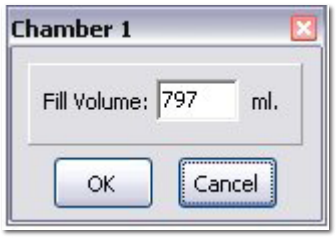
If **Auto** is checked, the chamber is filled up with the programmed amount of ml. Otherwise the chamber is filled until the 'fill up' button is clicked again.

Pressure is activated/ deactivated by clicking the 'off' button below or on the progress bar.
Red: The pressure is off.
Blue: The pressure is on

Zero force

Green: The chamber is in place.
Red: The chamber is missing or not positioned correctly.

When the reservoir bottles are refilled or filled for the first time, write the volume in the bottle in the window that pops-up.



If **All** is checked, the activation of 'Fill', 'Empty', 'Zero', 'Pressure' and 'Fill of chamber 1' will be applied to all the chambers.

3.2.3 Drop down menus

3.2.3.1 File menu

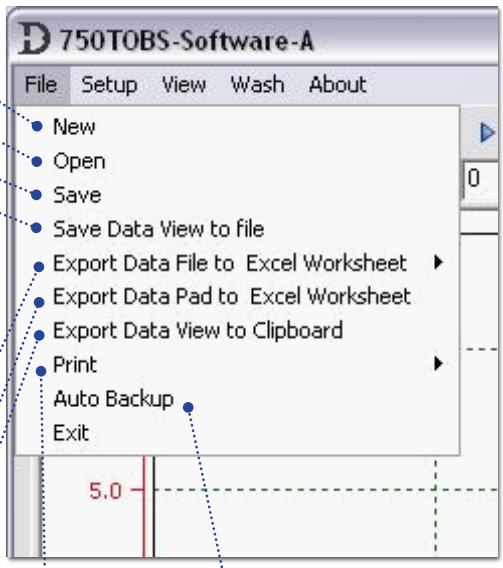
New:
Create a new file to begin a Data acquisition

Open:
Open an exciting file

Save:
Save the file

Save Data view to file:
Several image formats are supported for saving static images of the Data view. This is useful for exporting the image for use in an external reporting tool, or for other uses where a "snapshot" of the chart is needed. Supported image formats are:

- Enhanced Metafile Format (EMF)
- Windows Bitmap (BMP, RGB Encoded)
- Independent JPEG Group Format (JPEG, JPG)
- Portable Network Graphics Format (PNG, 16 million colors)



Export Data file to Excel worksheet:
See section 3.6.3

Export Data pad to Excel worksheet:
See section 3.6.2.

Export Data view to clipboard:
Same as Save Data view to file, except that the image is placed in the clipboard, and can be imported in any program using the paste command CTRL+V.

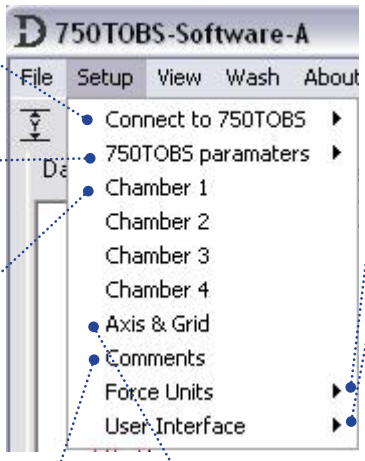
Auto backup:
Opens a new window where auto Backup is set.

Print->Print Data view:
Prints the Data view on the default printer.
Print->Print comments:
Prints the comments on the default printer.
Print->Print Data pad:
Prints the Data pad on the default printer.

3.2.3.2 Setup menu

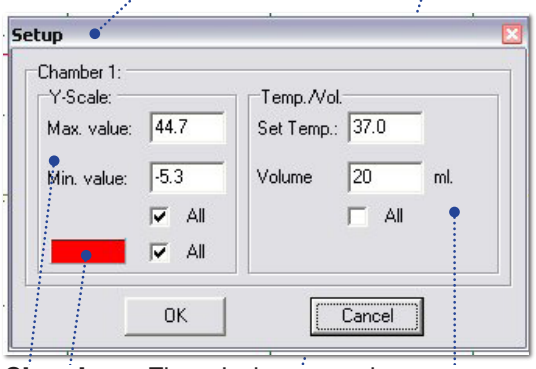
Connect to 750TOBS: Connect or disconnect the 750TOBS

750TOBS parameters: See sections 3.2.3.3 to 3.2.3.12

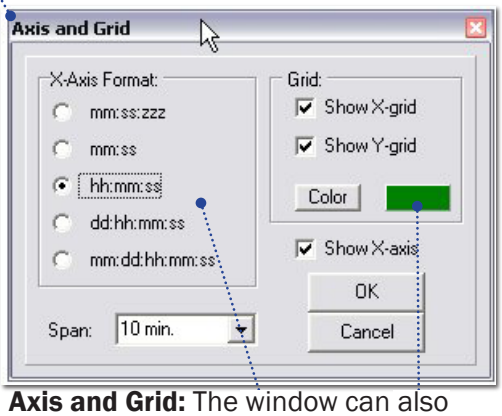


Force units: Choose between mN or gram

User Interface: There are two choices:
1. Controller. (described in Appendix 8. It is used when another software package is used for acquisition.)
2. Acquisition & Controller.



Chambers: The window can also appear by double clicking on the Y-axis.



Axis and Grid: The window can also appear by double clicking on the X-axis.

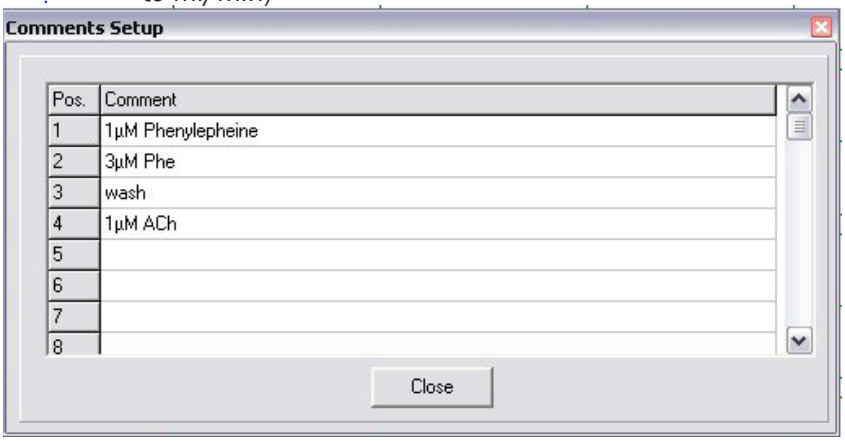
Y-axis setting

Y-axis' color

Change the settings for '**Set. Temp**'. and '**Volume**' in a choosen chamber. If '**All**' is checked, the changes will be applied to all four chambers. (In Constant Wash Mode, the Volume setting is changed to Flow, and units to ml/min)

X-axis' color

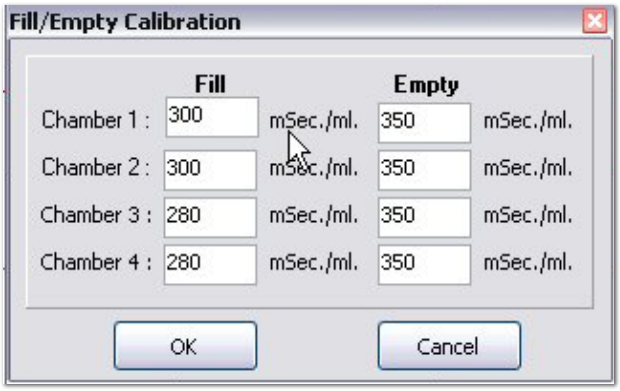
X-axis setting



Comments setup: Up to 20 default comments can be saved. The window can also appear by double clicking on the 'Comments input field'.

3.2.3.3 Fill/Empty calibration

Setup->750TOBS parameters ->Fill/Empty calibration



The amount of fluid filling the tissue chamber is time regulated. Calibration of each chamber is performed with the marked tissue chamber provided with the calibration kit (20 ml).

Adjust the numbers of each tissue chamber until the amount of fluid is correct, the numbers indicates msec/ml.
If the amount of fluid is to small, the number (msec/ml) must be higher. If the amount of fluid is to high the number (msec/ml) must be lower.

3.2.3.4 Pressure

Setup->750TOBS parameters->Pressure

Adjustment and on-line reading of pressure. This setting is normally **not** changed.

Actual pressure

'Act. Pressure' provides an on-line reading of the actual pressure in the reservoir bottles.

Set pressure

To set or reset the target pressure
(0.01- 0.999 Bar).
(Default: 0.400)

Update

'Update' provides an on-line reading of the actual pressure.

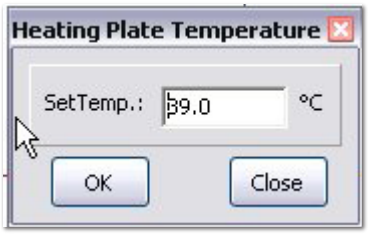
NOTE: When the pressure is reset press the small button that appears to the right of the Set Pressure Input field.
If the pressure is changed, the flow must be calibrated again, as described in paragraph 3.2.3.10

3.2.3.5 Heating plate temperature

Setup->750TOBS parameters->Heating plate temperature

Adjusts the temperature on the heating plate, on which the reservoir bottles are placed. The temperature here is chosen to be higher than the bath temperature to avoid gas formation in the lines and to correct for cooling during refilling of the chambers.

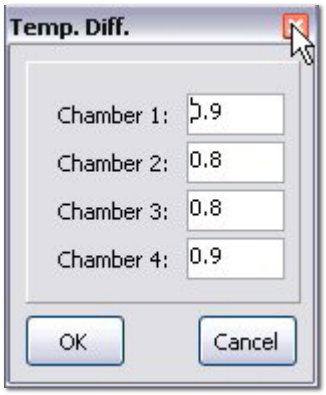
'Set Temperature' is used to set the set temperature of the heating plate for the reservoir bottles. (Default: 39 °C).
The Set Temp. is updated when clicking the OK button.
NOTE: To minimize the heating time one can benefit by pre-heating the buffer to 35 °C before filling the reservoir bottles for the first time in the day.



3.2.3.6 Temperature difference

Setup ->750TOBS parameters->Temperature difference

Adjustment between the actual temperature and target temperature.



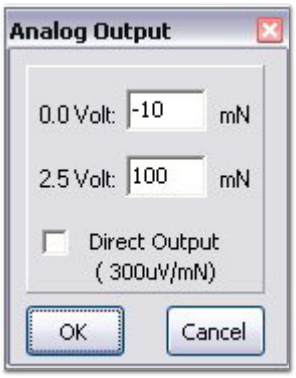
The Set temperature and Actual temperature may not be equal due to the ambient temperature in the lab. It is possible to compensate this with this setting.

Example: If the *Set.Temp* for Chamber 1 is 37.0 °C and the actual temperature measured in Chamber 1 is 36.2 °C . The *Difference Setting* for Chamber 1 must be 0.8.

3.2.3.7 Analog output

Setup ->750TOBS parameters-> Analog output

The output is from 0.0 to 2.5 Volt. (Resolution is 0.61 mV). In this window the span can be adjusted.
In the example above the span is set to 110 mN . The output is 0.0 Volt, if the force is -10 mN and 2.5 Volt if the force is 100 mN.



The internal sample frequency is 20 Hz on each channel. Any digital filtering in your data acquisition software, must be set above 20 Hz.

If the setting *Direct output* is choosen the settings above is not used. The output is then routed directly from the transducer :

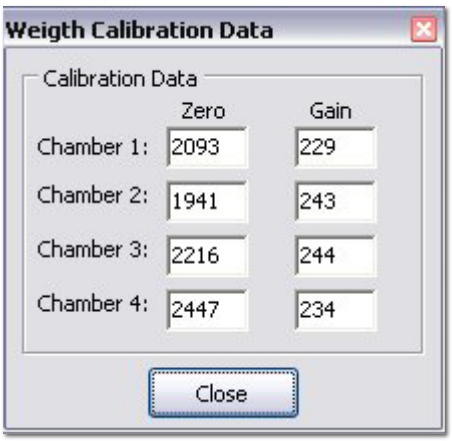
Offset: 2.5 Volt
Output: +/-300µV/mN

If *Direct output* is used the signal is not filtered and the output is only limited by the transducers response time, it is approximately 10 mS.

3.2.3.8 Weight calibration data

Setup ->750TOBS parameters->Weight calibration data

A read out of the *Force calibration data.* (Only for internal use, if there are some problems with a transducer).



3.2.3.9 Wash

Setup ->750TOBS parameters ->Wash

Programming of the wash sequence.
The wash sequence works by overflow.
In the example to the right the wash sequence is set to:
Wash 4 sec. every 1 min. 2 times, then wash 3 sec. every 1 min. 5 times.
If 'next wash' is not checked, there will only be one wash

3.2.3.10 Flow calibration

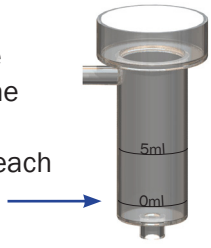
Setup ->750TOBS parameters->Flow calibration

Before this calibration is performed, the low flow shunt supplied in the calibration kit must be mounted on the tubing inside the reservoir bottle. Calibration of flow in Constant Wash mode is performed by the marked Tissue chamber provided with the calibration kit (5ml), (Optional for the 750TOBS, please contact DMT for purchase). On the Tissue chamber there are two marks used for the calibration. The calibration is done one chamber at the time.

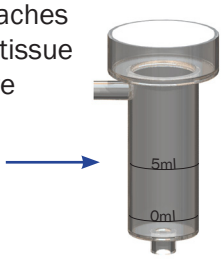
1. Turn off oxygenation.

2. Choose the chamber to calibrate.
   Click Start flow.

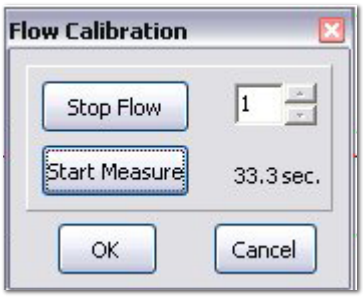
3. When the surface of the fluid reaches the bottom mark on the calibration tissue chamber, click the Start Measure button.Check this by looking at the line at eye level and look for the bottom of the fluid meniscus to reach the line!



4. When the surface of the fluid reaches the top mark on the calibration tissue chamber, click the Stop Measure button.



5. Click the OK button. Flow calibration for the chosen chamber is now finished.

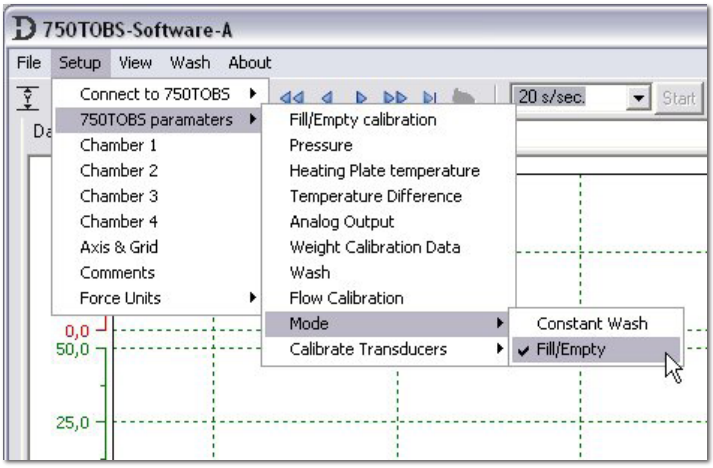


Repeat step 1-5 for all the chambers.

3.2.3.11 Mode select

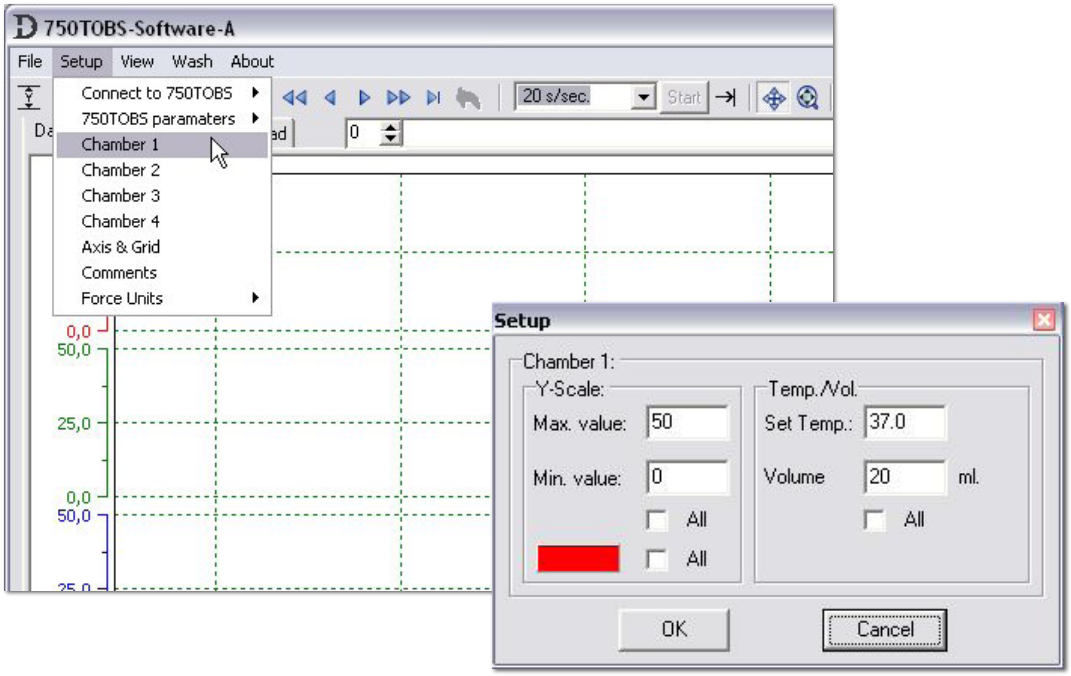
Setup ->750TOBS parameters-> mode ->

The 750TOBS has two different wash modes. Constant wash and Fill/empty.

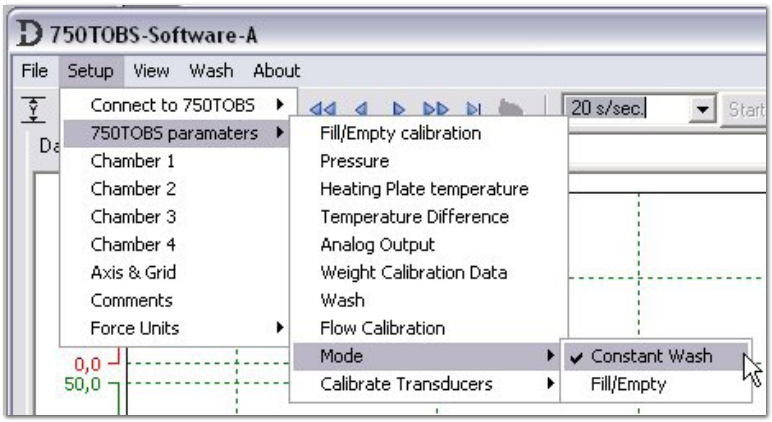


The classic way of changing solution in an organ bath system is to drain the entire bath and refilling it with fresh buffer via a Fill/empty routine. The advantage of this method is that the concentration of drug declining fast especially if this is repeated 3 times or more. The 750TOBS can do this automatically via the wash menu and the buffer comes from the pre-heated buffer reservoir in the system. Therefore compared to the classic approach, washing is easy, convenient and fast. A disadvantage of this fill/empty method is that the tissue is briefly suspended in air, without nutrients and stimulation, when used, needs to be suspended.

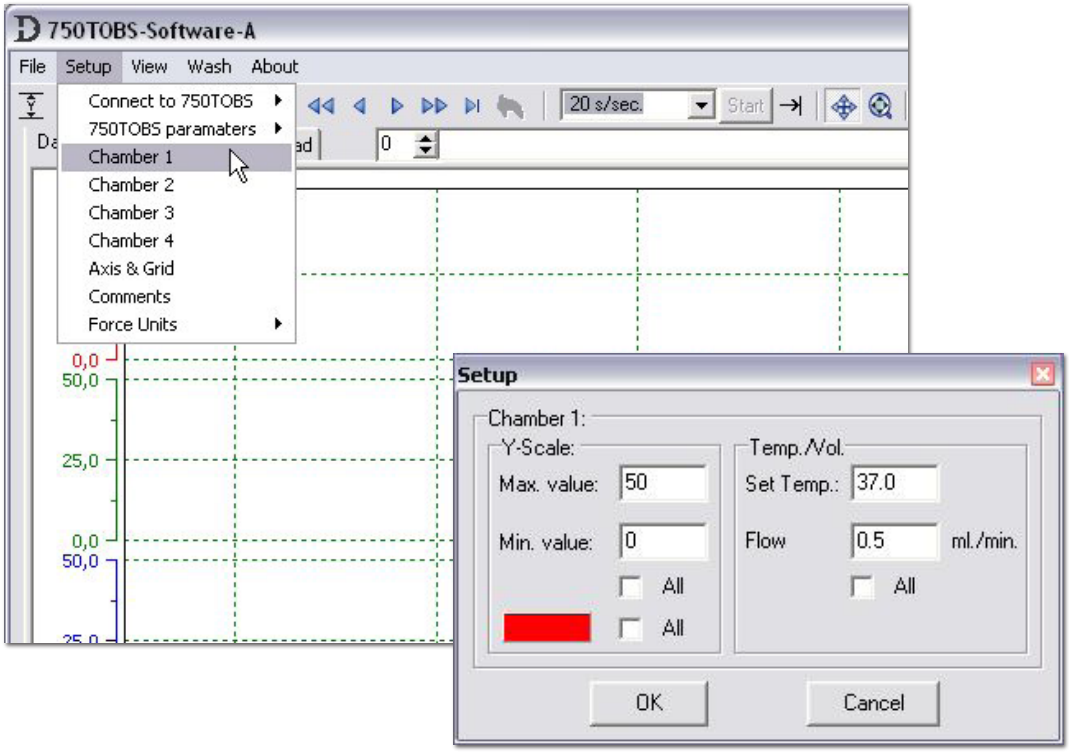
When the fill/empty mode is selected go to **Setup->Chamber 1-4 or double click on the chamber's y-axis** to adjust the settings.



A second new method in the 750TOBS is to perform a constant wash at a preset flow rate. In the 750TOBS the fresh warm buffer from the reservoir is filled from below and excess buffer is evacuated from the overflow on the top. The flow rate can be programmed from 0.5 - 5.0 ml/min . Although it may take a little more time to completely wash any drugs away, the advantages are that the tissue is never in air, stays in warm buffer and stimulation, if used, does not need to be interrupted. The user is encouraged to try both methods and based on own judgment determine what is best for the experimental conditions.



When the Constant wash mode is selected go to **Setup->Chamber 1-4 or double click on the chamber's y-axis** to adjust the settings.



Important: When the mode is changed, the shunt inside the bottle must be changed too.

The Constant wash mode requires the **Constant flow shunt** and Fill/Empty mode requires the **Fill/Empty flow shunt**.

Important: Make sure that the pressure is OFF before changing the shunts.

A warning noting that the pressure is turned off, is shown when the mode is changed.

Attention !
You are changing mode.
Please check that the right shunt is mounted in the bottle.
Please note that the pressure is removed.
Close

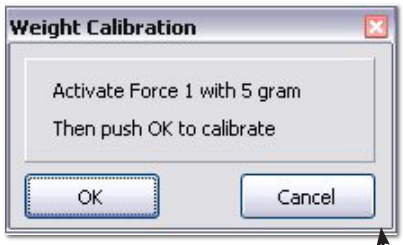
Constant flow shunt
(Hypodermic needle 0,4 x 40mm)

Fill/Empty flow shunt

Accessories, please contact DMT for purchase.

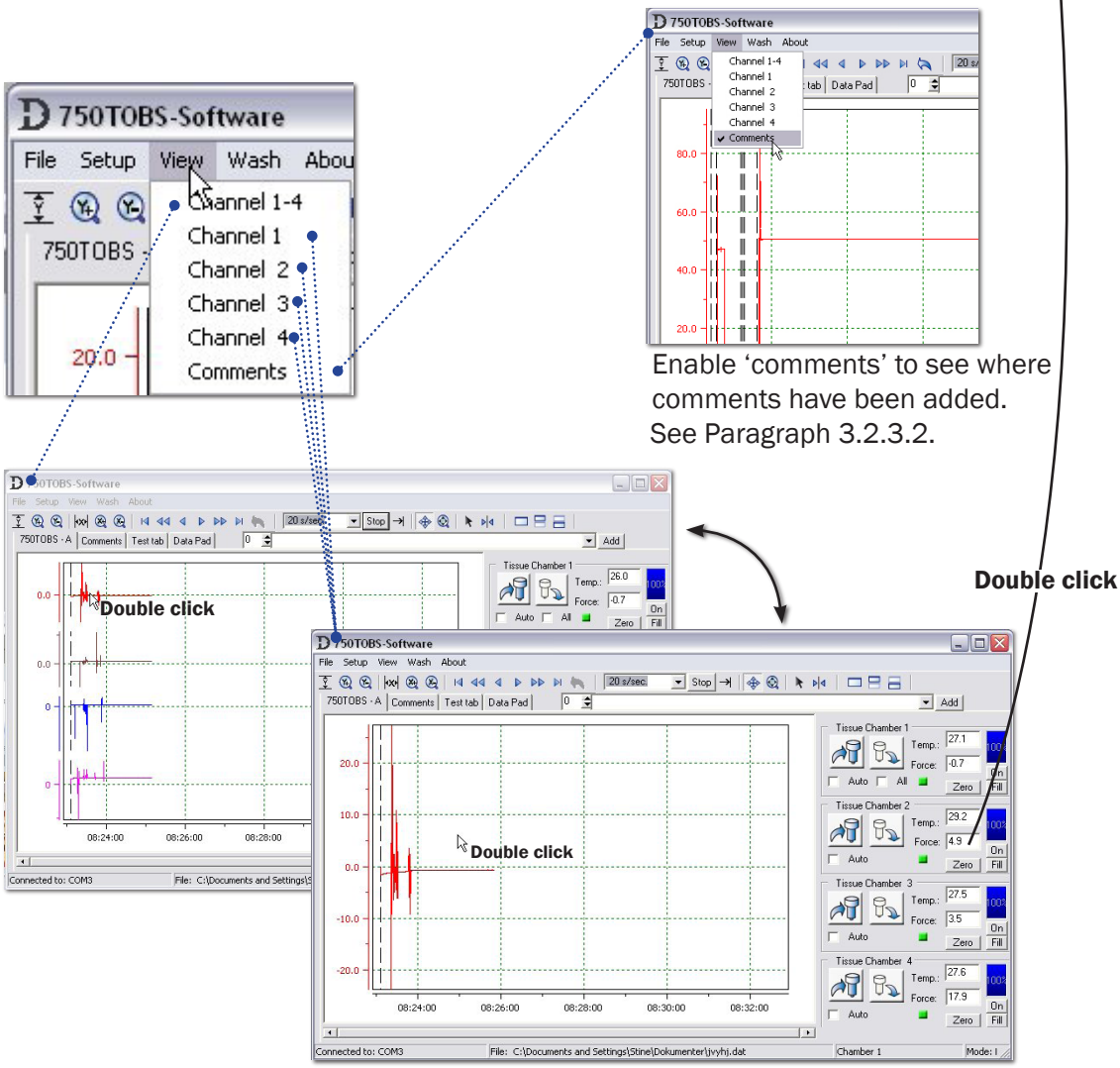
3.2.3.12 Weight calibration

By activating **Setup ->750TOBS parameters-> Calibrate transducers -> Chamber X** a message appears as shown to the right.
Place the 5g weight on the transducer. Wait until the relative force reading is stable. Click OK.
The force applied on the force transducer should mimic the stretch created by the contraction of a mounted ring preparation.



3.3.1 View

The Dataview can be set to display all four channels or one single channel.

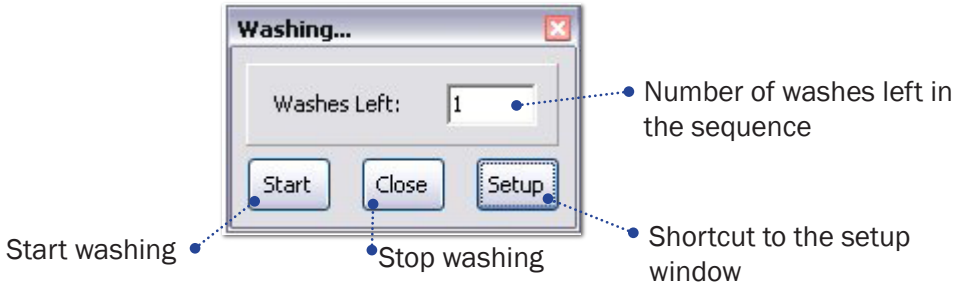


Enable 'comments' to see where comments have been added. See Paragraph 3.2.3.2.

Double clicking on the channel will also result in shifting between one or four channels

Double clicking on the Fprce value will open the 'Weight calibration' window.

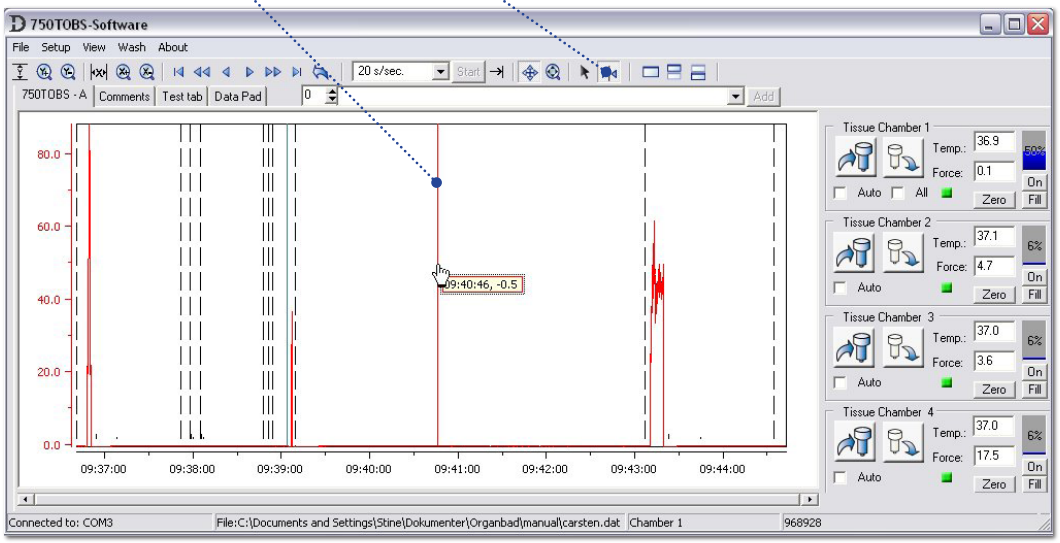
3.4.1 Wash



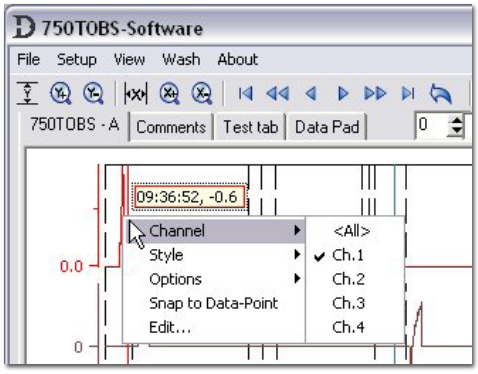
3.5.1 The Dataview area:

3.5.2 Reading data

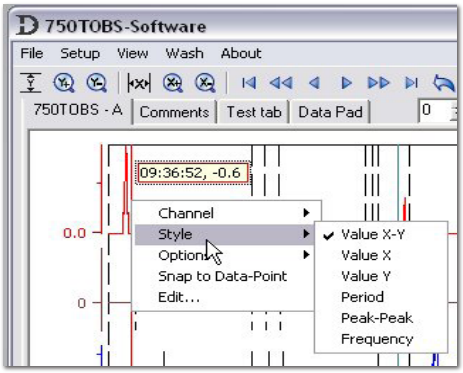
Activate the Cursor button . A line and a box with the readings will appear on the datasheet. Move the cursor by holding the left mouse button while moving the mouse along the curve.



The automatic data scrolling on the X-axis is stopped, when the Select Button or the Cursor button has been activated.
The automatic scrolling is started again by activating the Resume button .



Change the channel on which the data is read by right clicking on the cursor line



Change the style on which the data is read by right clicking on the cursor line

3.5.3 Comments tab

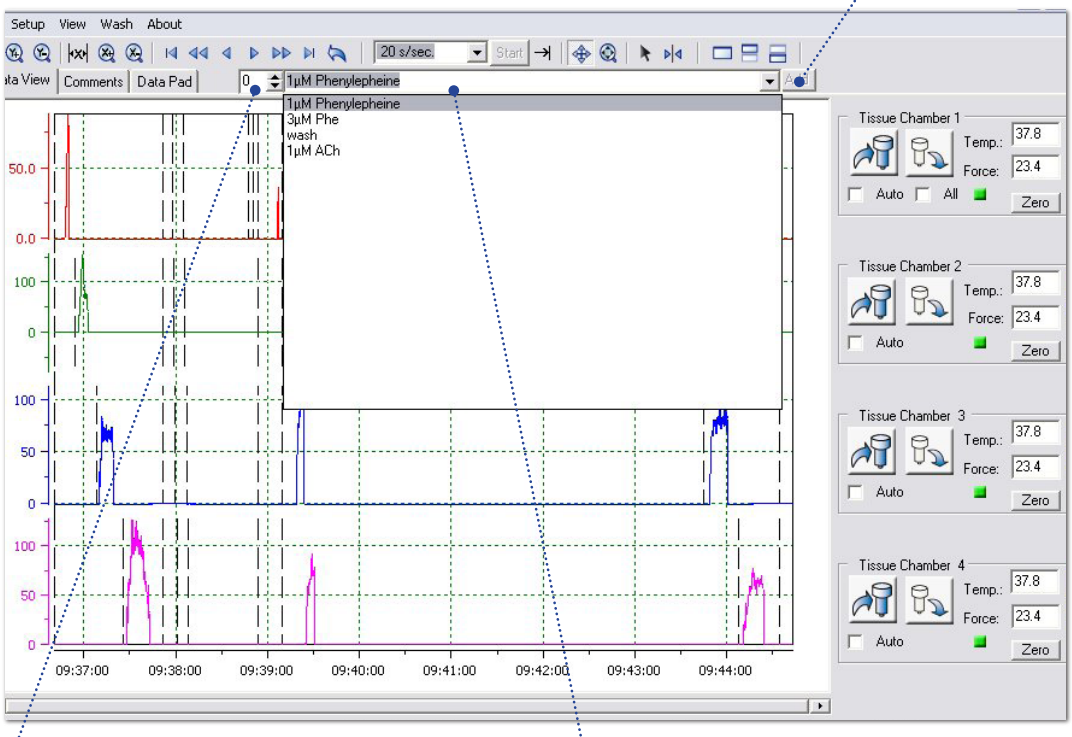
Adding comments to the Dataview

During the data acquisition, comments can be added eg. when the force is zeroed or when a drug is added. A dashed line on the datasheet indicates where a comment has been added. The dashed line can be made invisible by removing the check mark next to 'Comments' in the 'View' dropdown menu.

To add a comment: Choose which channel the comment has to belong to. Click on the 'Add' button - choose a default comment or write a new comment in the text box.

Important: Comments can only be added when acquisition is started.

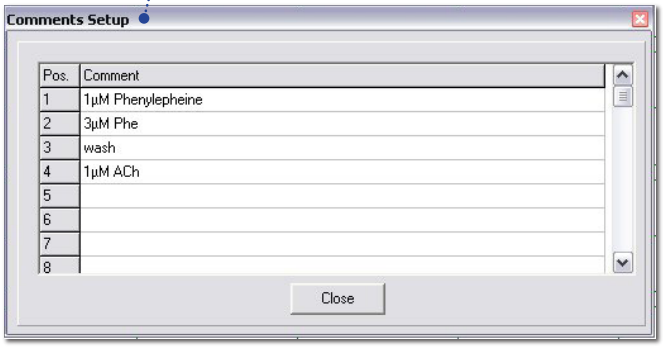
Click on 'Add' to save a comment.



Choose which channel to comment on (0= all channels)

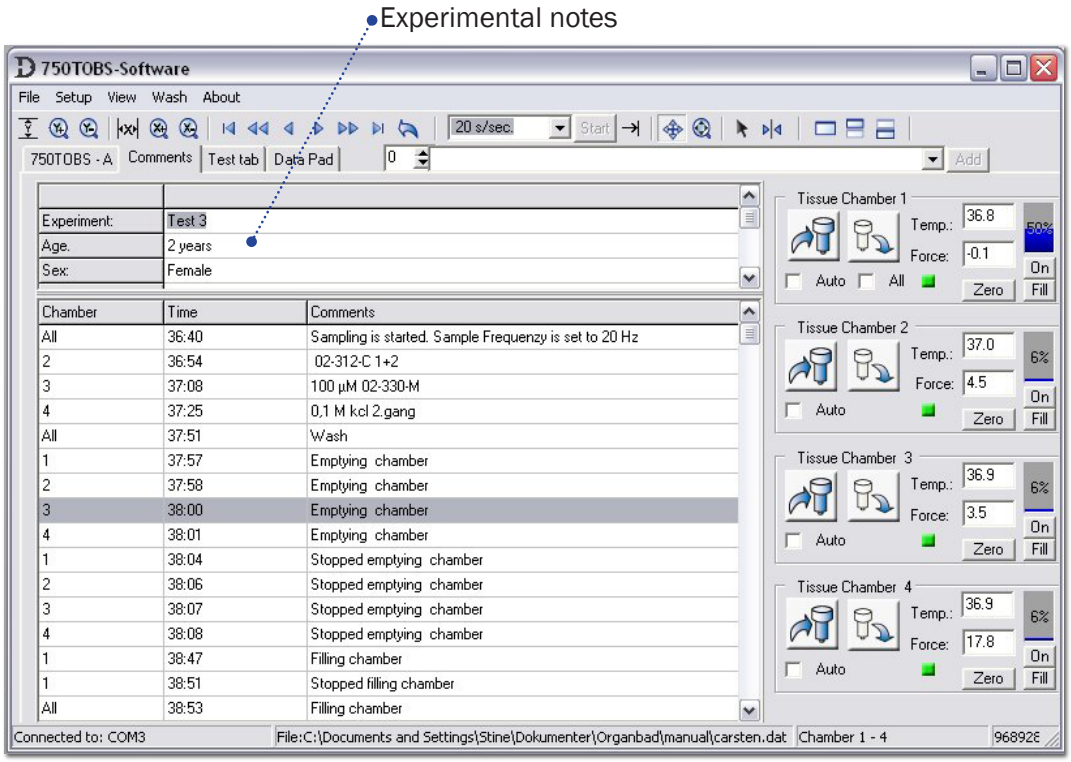
Write or choose a default comment.

Double clicking on the comment input field opens the 'Comments Setup'. 20 default comments can be stored here.



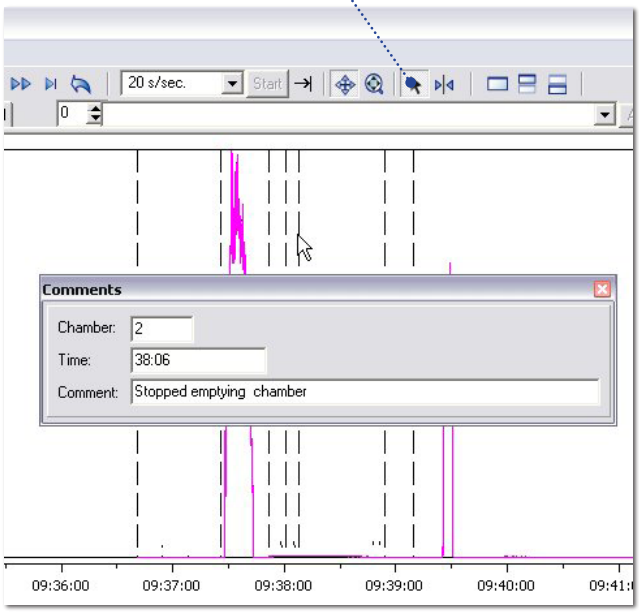
3.5.4 Viewing comments

All added comments can be seen in the comment sheet when clicking on the 'Comments tab'. By double clicking on a comment, the view changes to the Dataview tab, to the place where the comment was added. The dashed line will flash for 5 seconds.


Experimental notes
750TOBS-Software
File Setup View Wash About
20 s/sec.
Start
750TOBS - A
Comments
Test tab
Data Pad
Add
Experiment: Test 3
Age. 2 years
Sex: Female
Chamber | Time | Comments
All | 36:40 | Sampling is started. Sample Frequenzy is set to 20 Hz
2 | 36:54 | 02-312-C 1+2
3 | 37:08 | 100 µM 02-330-M
4 | 37:25 | 0,1 M kcl 2.gang
All | 37:51 | Wash
1 | 37:57 | Emptying chamber
2 | 37:58 | Emptying chamber
3 | 38:00 | Emptying chamber
4 | 38:01 | Emptying chamber
1 | 38:04 | Stopped emptying chamber
2 | 38:06 | Stopped emptying chamber
3 | 38:07 | Stopped emptying chamber
4 | 38:08 | Stopped emptying chamber
1 | 38:47 | Filling chamber
1 | 38:51 | Stopped filling chamber
All | 38:53 | Filling chamber
Tissue Chamber 1
Temp.: 36.8
Force: -0.1
Auto All Zero Fill On
Tissue Chamber 2
Temp.: 37.0
Force: 4.5
Auto Zero Fill On
Tissue Chamber 3
Temp.: 36.9
Force: 3.5
Auto Zero Fill On
Tissue Chamber 4
Temp.: 36.9
Force: 17.8
Auto Zero Fill On
Connected to: COM3
File:C:\Documents and Settings\Stine\Dokumenter\Organbad\manual\carsten.dat
Chamber 1 - 4

A comment can also be seen in the Dataview area:

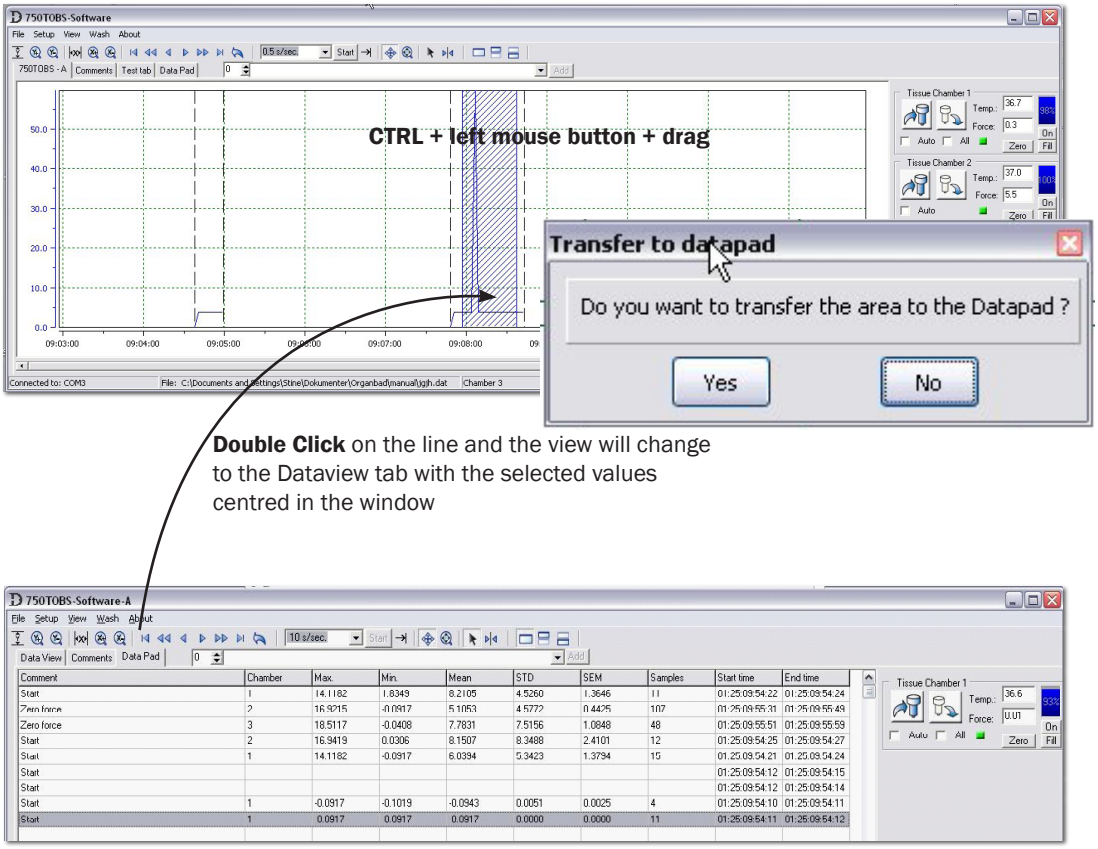
Choose the select tool and hold the cursor above a comment line. Left click and a new window opens with data for the selected comment.



3.6.1 Transfer Data to the Datapad

A specific data area in the channel can easily be transferred to datapad.

Select the data to be transferred by holding the CTRL key while dragging the mouse over the area where the data needs to be collected. When the left mouse button is released a 'Transfer to datapad' window appears. Click 'Yes'.
The data can now be found in the 'DataPad' tab


750TOBS-Software
CTRL + left mouse button + drag
Transfer to datapad
Do you want to transfer the area to the Datapad ?
Yes
No
Double Click on the line and the view will change to the Dataview tab with the selected values centred in the window
750TOBS-Software-A

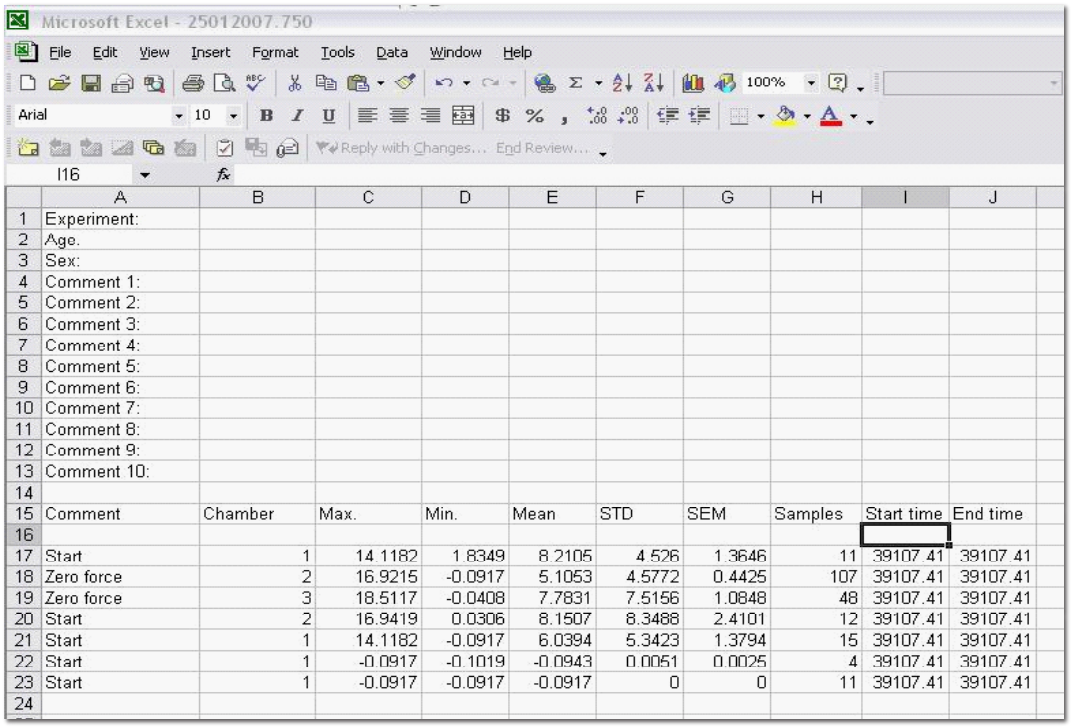
The chosen data from the Data view is transferred to the Data Pad as:

Comment: The comment is the nearest comment to the left of the transferred data.
Chamber: The chamber where the data is captured from.
Min.: The minimum value of the transferred data.
Max.: The maximum value of the transferred data.
Mean: The mean value of the transferred data.
STD: The Standard Deviation of the transferred data.
SEM: The Standard Error of Means of the transferred data.
Samples: The number of samples in the transferred data.
Start time: The start time of the transferred data.
End time: The end time of the transferred data.

3.6.2 Export a Datapad file to an Excel worksheet

All the Data from the DataPad can be exported to a *.750 file format (choose text, txt format in excel) that can be opened in an Excel Worksheet.

Open the *.750 file in a Microsoft Excel worksheet.



Microsoft Excel - 25012007.750

File Edit View Insert Format Tools Data Window Help

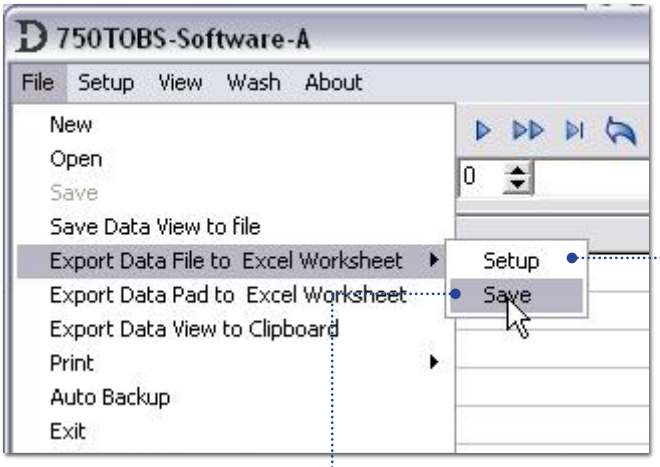
Arial 10

I16

	A	B	C	D	E	F	G	H	I	J
1	Experiment:									
2	Age:									
3	Sex:									
4	Comment 1:									
5	Comment 2:									
6	Comment 3:									
7	Comment 4:									
8	Comment 5:									
9	Comment 6:									
10	Comment 7:									
11	Comment 8:									
12	Comment 9:									
13	Comment 10:									
14										
15	Comment	Chamber	Max.	Min.	Mean	STD	SEM	Samples	Start time	End time
16										
17	Start	1	14.1182	1.8349	8.2105	4.526	1.3646	11	39107.41	39107.41
18	Zero force	2	16.9215	-0.0917	5.1053	4.5772	0.4425	107	39107.41	39107.41
19	Zero force	3	18.5117	-0.0408	7.7831	7.5156	1.0848	48	39107.41	39107.41
20	Start	2	16.9419	0.0306	8.1507	8.3488	2.4101	12	39107.41	39107.41
21	Start	1	14.1182	-0.0917	6.0394	5.3423	1.3794	15	39107.41	39107.41
22	Start	1	-0.0917	-0.1019	-0.0943	0.0051	0.0025	4	39107.41	39107.41
23	Start	1	-0.0917	-0.0917	-0.0917	0	0	11	39107.41	39107.41
24										

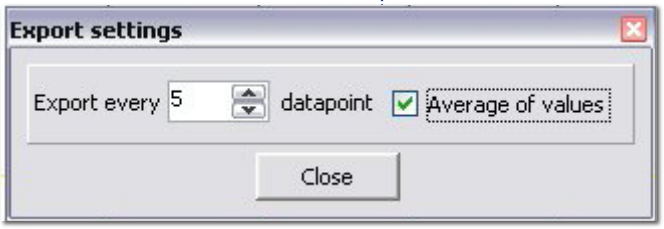
3.6.3 Export a Data file to an Excel Worksheet

All the Data from the Dataview can be exported to a *.750 file format that can be opened in an Excel Worksheet.



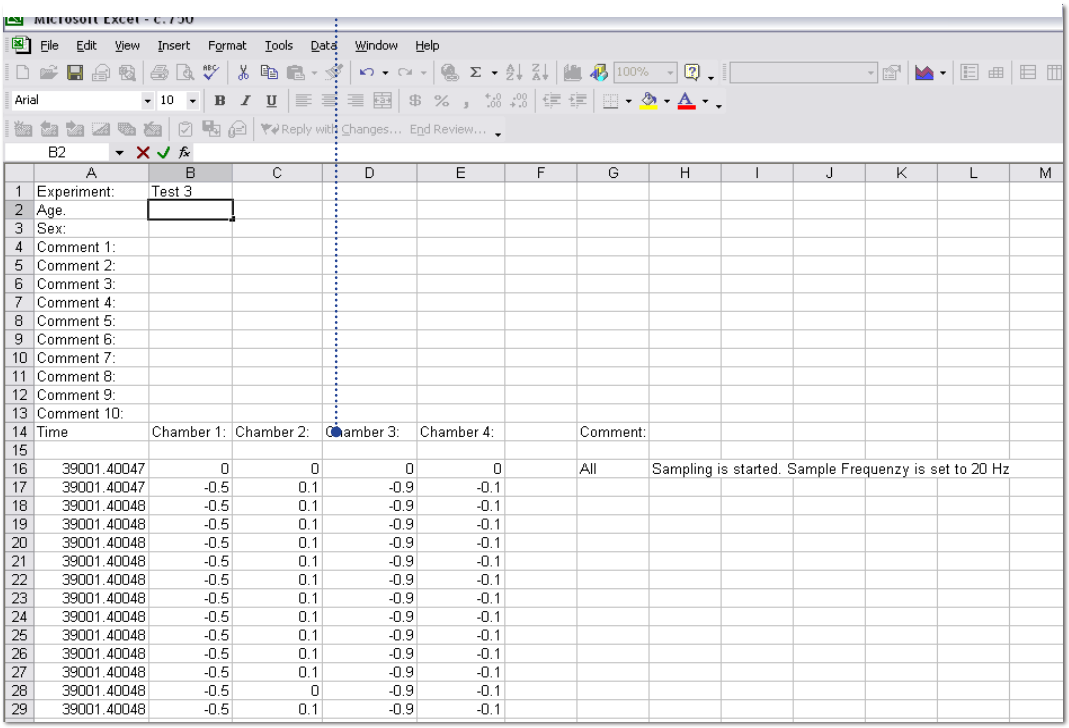
Click here to open the Export settings dialogue

In the dialogue box, the resolution of the data is defined. In this example every 5th. datapoint is saved as an average value of 5 datapoints.



File->Export Data File to Excel Worksheet-> Save, opens a standard Save file dialogue window. Give the file a name and click save.

Open the *.750 file in a Microsoft Excel worksheet.



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Experiment:	Test 3											
2	Age.												
3	Sex:												
4	Comment 1:												
5	Comment 2:												
6	Comment 3:												
7	Comment 4:												
8	Comment 5:												
9	Comment 6:												
10	Comment 7:												
11	Comment 8:												
12	Comment 9:												
13	Comment 10:												
14	Time	Chamber 1:	Chamber 2:	Chamber 3:	Chamber 4:		Comment:						
15													
16	39001.40047	0	0	0	0		All	Sampling is started. Sample Frequenzy is set to 20 Hz					
17	39001.40047	-0.5	0.1	-0.9	-0.1								
18	39001.40048	-0.5	0.1	-0.9	-0.1								
19	39001.40048	-0.5	0.1	-0.9	-0.1								
20	39001.40048	-0.5	0.1	-0.9	-0.1								
21	39001.40048	-0.5	0.1	-0.9	-0.1								
22	39001.40048	-0.5	0.1	-0.9	-0.1								
23	39001.40048	-0.5	0.1	-0.9	-0.1								
24	39001.40048	-0.5	0.1	-0.9	-0.1								
25	39001.40048	-0.5	0.1	-0.9	-0.1								
26	39001.40048	-0.5	0.1	-0.9	-0.1								
27	39001.40048	-0.5	0.1	-0.9	-0.1								
28	39001.40048	-0.5	0	-0.9	-0.1								
29	39001.40048	-0.5	0.1	-0.9	-0.1								

The data in column A is in a data/time format used in Microsoft Excel. It can be formatted in the following way: Mark column A, right click, choose Format Cells, choose the Number tab, choose Time. Now it is possible to chose time format.

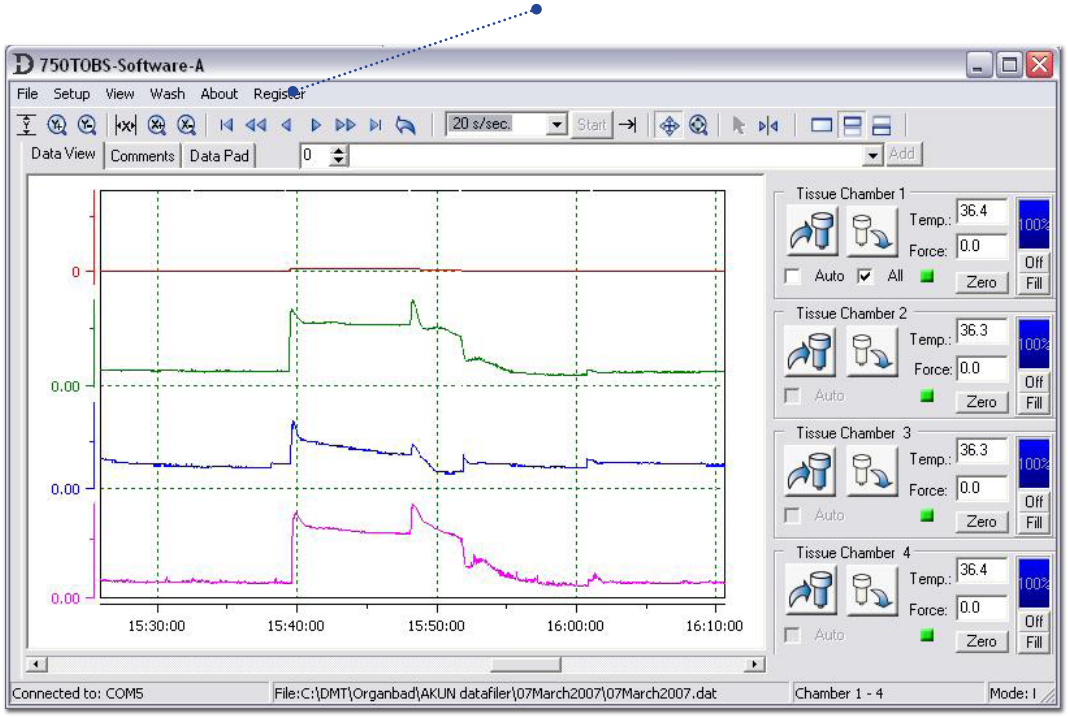
Note: To show the data/time correct in column A, it is important that the setting under Control panel->Regional options->General is set to English.

3.4.2 About

In the About box, software, firmware and hardware ID, for the 750TOBS is shown. There are also links to DMT support and website.

3.4.3 Register

If you want to register the software, click Register and your Web browser will open. It is now possible to register at DMT´s website.



Chapter 4 - The Tissue organ bath unit

Chapter 4 contains a complete explanation of how to calibrate and maintain the Tissue Organ Bath units to ensure the equipment is always performing to the highest standard.

4.1 The first weight calibration

Prior to the shipment of the Tissue organ bath 750TOBS system, it has gone through two days of continuous testing including a final weight calibration. However in order to ensure that the Tissue organ bath is working at highest performance, DMT recommends that a new weight calibration is performed before starting to use the Tissue organ bath system. The calibration is performed through the “calibration menu” using the appropriate weight according to gain setting. As default the system is set with a gain range of -20/+200 mN and the calibration is performed using a 5g weight. (fig. 4.1)

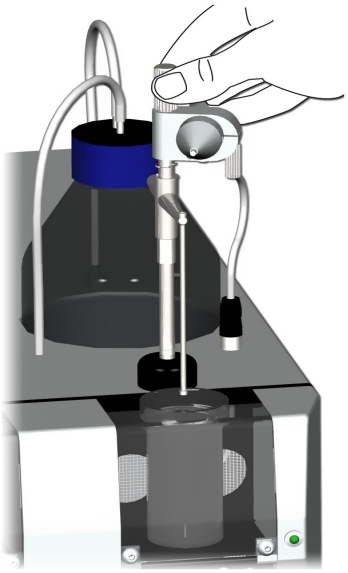
The weight calibration procedure is described in detail in Section 3.2.2.12.

Figure 4.1 *Transducer*

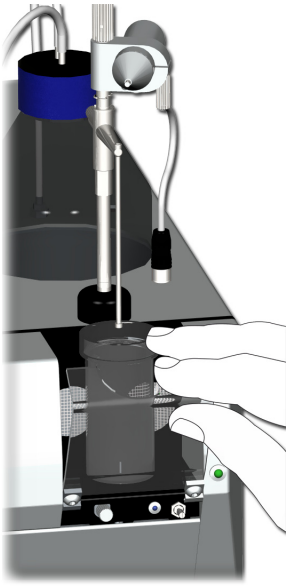
As a part of the general maintenance of the Tissue organ bath system, DMT recommends that the organ bath is weight calibrated at least once every month. DMT also recommends that the bath is weight calibrated every time the system has been moved or has not been used for a long period of time.

4.3 Tissue chamber handling

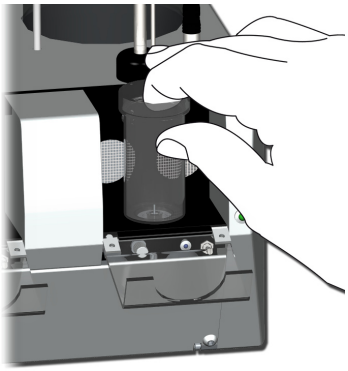
The following is a description of how to handle the tissue chambers and the different kinds of sample holders available.



Placing the glass:
1. Move the transducer upwards. Make sure the sample holder is free of the tissue chamber.



2. Open the chamber door

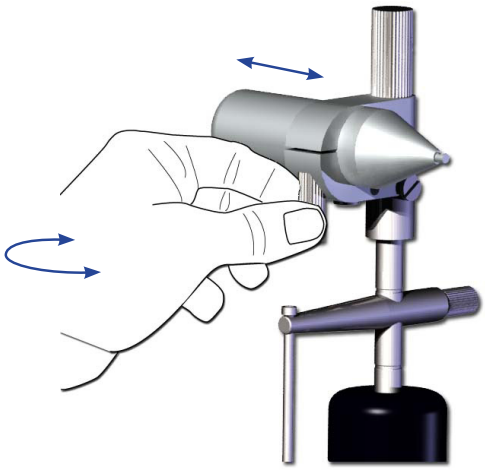


3. Place the pipe stub at the bottum of the chamber inside the chamber disc. Gently place the pipe stub situated on the side inside the heating compartment.

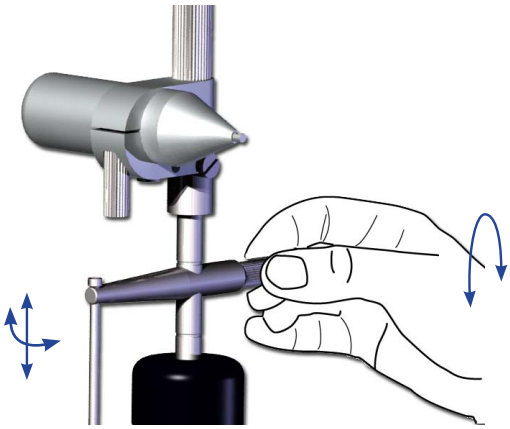
Removing the glass: *Carefully pull the top of the chamber outwards a few millimeters until the chamber is free to move upwards.*

4.3.1 Adjusting the transducer

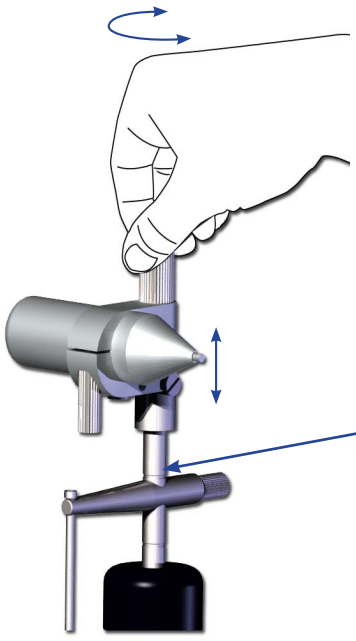
When tissue is placed in the sampleholder do a course adjustment on the tissue holder to fit the tissue just between the holder and the transducer without stretching it. **With the tissue submerged in the buffer set the force to 0**. Then use the micrometer positioner to stretch the tissue to the disired resting tension.



Horizontal adjustment of the transducer.
Turn the micrometer screw clockwise to unlock the transducer. When unlocked it is possible to move the transducer horizontially, (With the other hand). Turn counter clockwise to lock .



Course adjustment of the lower tissue holder.
Turn the micrometer screw clockwise to unlock the transducer. When unlocked it is possible to move the tissue holder up, down, left and right. Turn counter clockwise to lock .



Vertical fine adjustment and stretching the tissue.
Turn the micrometer screw clockwise to lower the transducer. Turn anti clockwise to increase the distance .

The marked line is the lowest position of the transducer. The highest position is 20mm above the line.

4.4 Sample holders

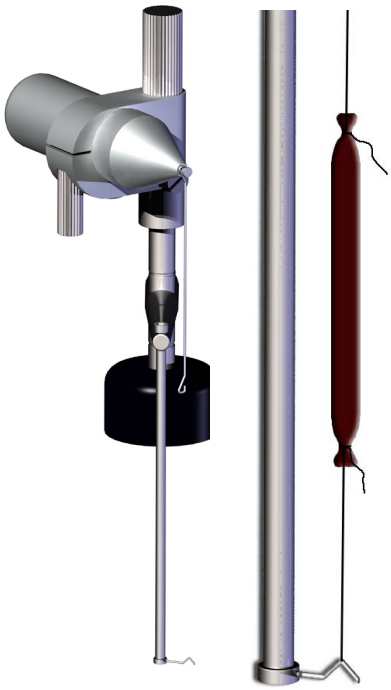


Figure 4.4.1 *Triangle hooks.*

Figure 4.4.2 *Closing clips (optional)*

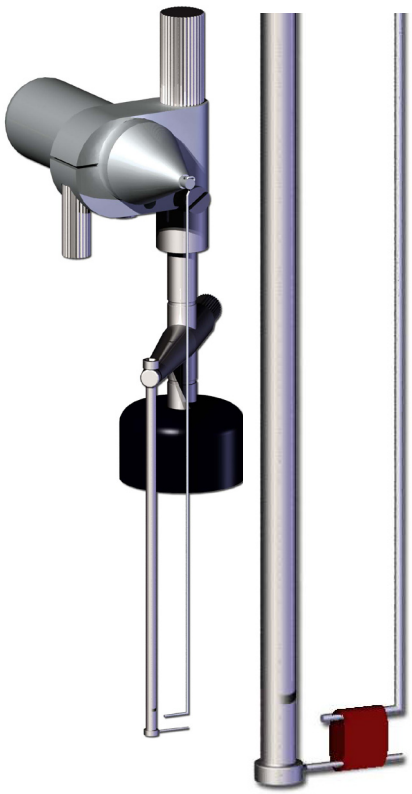


Figure 4.4.3 *Pins (optional)*

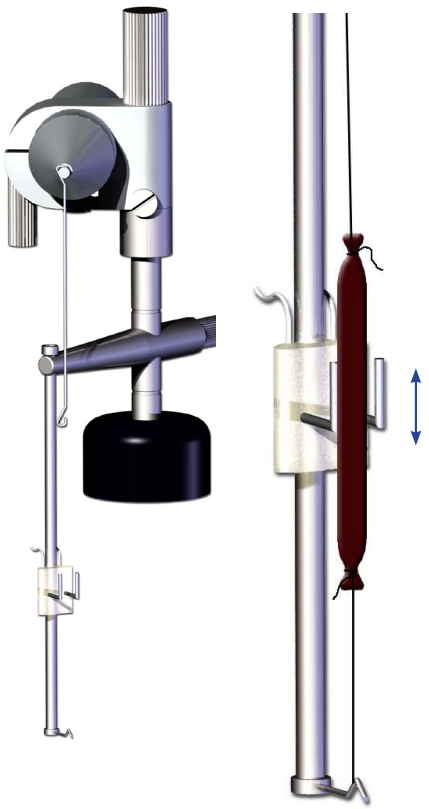


Figure 4.4.4 *Triangle hooks w/ stimulation electrodes (optional)*

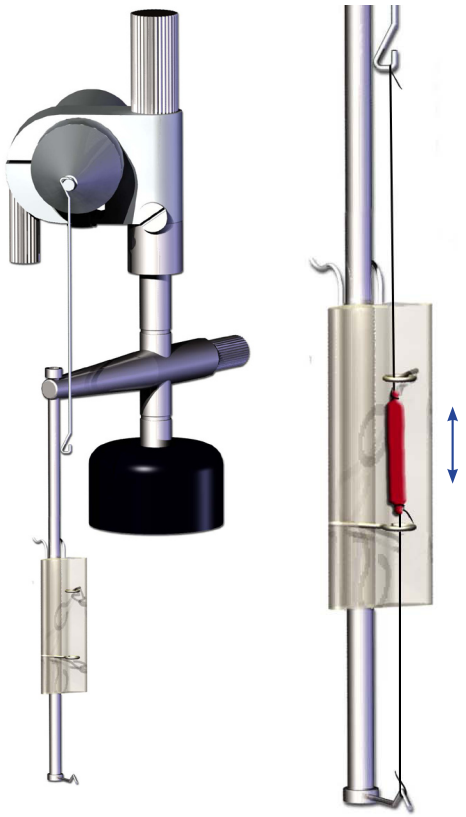


Figure 4.4.5 *Triangle hooks w/circular stimulation electrodes (optional)*

4.5 Tissue organ bath maintenance

The Tissue organ bath system is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and that the instructions are followed at all times.

Important: It is VERY important that the tubing being used is the original tubing from DMT. The quality, diameter and length are VERY important in order to insure the correct draining and filling of the chamber.

4.6 Tissue organ bath cleaning

DMT strongly recommends that the tissue bath chamber and surroundings should be cleaned after each experiment.
After a "normal" experiment use the following procedure to clean the tissue bath chamber, tubing and tissue holders.

1. Turn Pressure OFF
2. Use clean Schott Duran bottles
3. Fill with distilled water
4. Turn Pressure ON
5. Rinse the silicone tubing with double distilled water by activating the Fill & Empty Control several times.

Figure 4.6.1
Schott Duran bottle

NOTE: Only works when a tissue chamber is in place

6. Change the tubing after use of unknown or toxic chemicals, or if any kind of hydrophobic reagent has been used.
7. Carefully dismount the tissue bath chamber and perform a normal glass wash.
8. If aggressive solution or drugs have been used it is possible to disconnect the temperature probe (Fig. 4.6.1a) and take out the chamber disc. Clean both with double distilled water, and dry with a paper towel.

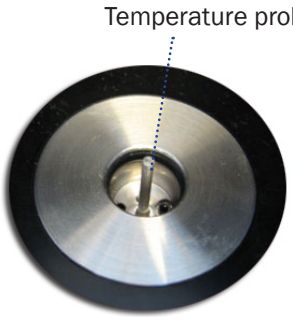


Figure 4.6.1a
Chamber disc and temp. probe

Important Note:
In exceptional cases it may be necessary to demount the sample holders for cleaning to make sure that all surfaces are cleaned.

Chapter 5 - Tissue organ bath accessories and spare parts

This chapter contains a complete list of equipment needed to setup an Organ bath system. In addition the chapter contains a list of special Tissue organ bath 750TOBS accessories and spare parts.
Besides the main focus on development and manufacturing, DMT has specialised in offering our customers first class laboratory equipment needed for a lab operating a tissue bath set up at very competitive prices. Please contact DMT Sales Department for further product information and prices.

5.1 General Tissue organ bath equipment

This list contains a complete checklist of laboratory equipment needed when setting up a Tissue organ bath system:

- **Dissection stereo microscope**
  DMT recommends the Zeiss Stemi 2000 stereo microscope.

- **Dissection scissors** DMT Item # DS-1000
  DMT recommends the Geuder G-19745 8 cm straight scissors.

- **Dissection and mounting forceps** DMT Item # DF-3000
  DMT recommends the Dumont medical No 5 and 55 tip (0.10 mm x 0.06mm).

- **Pipettes**
  DMT recommends CappAero™ pipettes.

- **Schott Duran glass bottle 1000ml** (other bottles may not fit)

- **Vacuum pump**
  DMT recommends a membrane vacuum pump having a suction volume of at least 11 litres per minute.

- **Dissection petri dish** DMT Item # PD-2000
  DMT recommends a ~9 cm glass Petri dish coated with a 5 mm Sylgard polymer layer.

- **Grease** DMT Item # HVG-1000

5.2 Tissue organ bath system accessories

This list contains a list of special accessories available for the Tissue organ bath system – Model 750TOBS:

- **ADI PowerLab data acquisition system**
  Including Chart data acquisition and analysis software.

- **Standard PC system** DMT Item # 80150

- **Constant flow shunt kit** DMT Item # 80150

- **CS200 4 channel Current stimulator** DMT Item # CS-200

5.3 Tissue organ bath 750TOBS system spare arts

This list contains a complete list of standard available spare parts for the Tissue organ bath 750TOBS. For parts not listed in this Chapter or for special parts, which may need to be custom made, please contact DMT for further information.

- **Force transducer**

- **Calibration Kit**

- **Tissue bath chamber size 5 ml** DMT Item # 75105
  10 ml DMT Item # 75110
  20 ml DMT Item # 75120
  50 ml DMT Item # 75150

- **Mounting hooks**

- rings Pins - L-shape - 4 sets DMT Item # 75300
- rings (EFS) Pins - L-shape w stimulation electrodes - 4 sets DMT Item # 75310
- bladder Closing clips - 4 sets DMT Item # 75320
- bladder (EFS) Closing clips - 4 sets w stimulation electrodes DMT Item # 75330
- strips Triangle hooks (standard) - 4 sets DMT Item # 75340
- strips (EFS) - 4 sets Triangle hooks (standard) w stimulation electrodes - 4 sets DMT Item # 75350

- **Tubing kit** DMT Item # 75400

Appendix 1 - Service Check

A Tissue organ bath system working at optimal performance is extremely important for success. To make sure that our customers always are dealing with first class Tissue organ bath systems, DMT offers a Service check at a very favourable price.

The Tissue organ bath system Service check includes a complete separation for inspection of all mechanical and electronic parts. The Tissue organ bath is then reassembled, adjusted and finally all electronic and mechanical parts are tested.

Please note that the service does not include replacement of transducers or any other needed spare parts. Please contact DMT for information about prices.

Appendix 2 - Shipping instructions

If the Tissue organ bath system needs to be returned for service or repair, please read the following shipping instructions very carefully.

Before you start packing the Tissue organ bath system, please remember that you are dealing with very delicate equipment and therefore care must be taken. DMT recommends that each part of the system be wrapped individually (i.e. with bubble wrap) and placed together in a large box (preferably the box you once received the tissue bath system in). Place the wrapped items in the middle of the box and fill out the surroundings with chips of expanded polystyrene.

Important:
Before closing the box, make sure that no enclosed items can be shaken around, as transport by road or air from time to time is quite rough.

Address the box to:
DMT A/S
Skejbyparken 152
DK-8200 Aarhus N
Denmark

Make sure that all four sides of the box are marked “fragile” or similar. Make an indication on the top of the box that it contains goods returned for repair/service.

Customers outside the EC must further enclose a proforma invoice stating that the box contains goods being returned for repair or service.

If arranging transportation through a courier, please keep in mind the high value of the Tissue Organ Bath System and that a standard insurance provided by the courier in most cases is insufficient to cover damage or loss of the system. In most cases an additional insurance coverage is needed.

Appendix 3 - System Specifications

Specifications:

Chamber

Chamber size(s):	5, 10, 20 or 50 ml
Chamber material:	Glass
Chamber heating:	Heated air circulation (built-in - patent pending)
Temp. range:	<50°C
Temp. resolution:	0.1°C
Temp. stability:	+/- 0.1°C
Chamber suction:	Manual or automatic via software
Chamber aeration:	Individually via precision needle valve(s)
Chamber filling:	Manual / automatic via software
Chamber filling method:	Drain/fill or constant wash

Transducer

Force range:	+/- 200 mN
Output resolution:	0.1 mN (using filtered signal)
Transducer calibration:	Semi automatic (via software)

Reservoir

Reservoir volume:	4 x 800 ml
Reservoir heating:	Electronically (built-in)
Inlet pressure:	Min. 2.0 bar - max 8.0 bar
Safety valve:	10 bar
Temp. range:	<50°C
Temp. resolution:	0.1°C
Temp. stability:	+/- 0.2°C

Analogue output(s):	4 x BNC / 2.5V FS (filtered signal)
Data communication:	USB (2.0)
Voltage:	110 - 240V / 50 - 60 Hz

Appendix 4 - Fuse changing

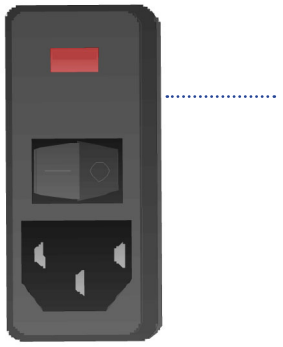
The main fuse of the Tissue organ bath system is placed inside the power inlet on the 750TOBS. If the fuse blows it is easily changed using the following procedure.

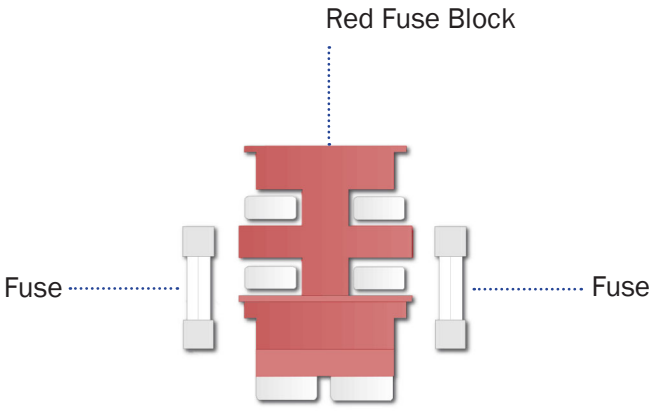
Important:
When a fuse blows and needs to be changed, it is imperative that the replacement fuse is equal to the one blown.
The 750TOBS system uses: T2A / 250V, 6.3 x 32 mm

DMT recommends that both fuses in the fuse block are changed at the same time, as it can be difficult to determine which fuse is blown.

To replace the fuses:

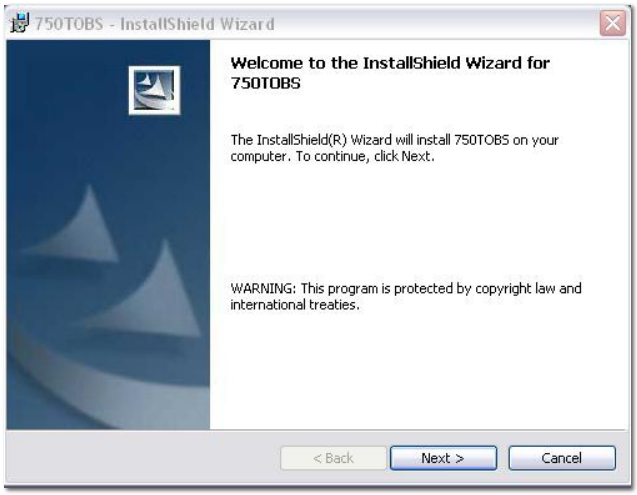
1. Use a small screwdriver to open the voltage selector block.
2. Remove the red fuse block.
3. Remove the existing fuses.
4. Insert the new fuses.
5. Replace the fuse block back into the voltage selector block
   Note: ensure that the correct voltage for your country is displayed.





Appendix 5 - Software installation

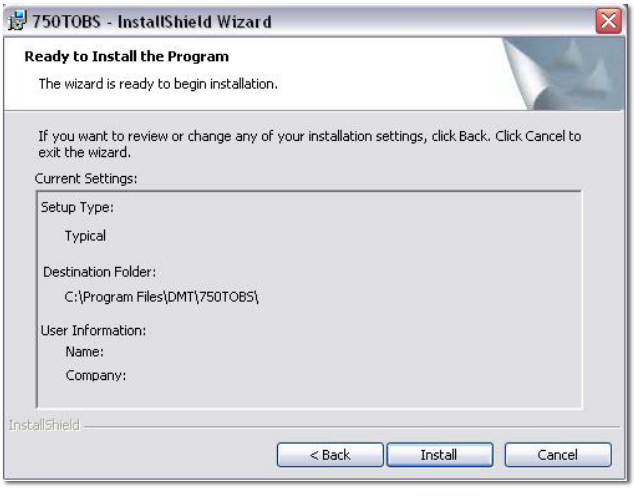
Insert the 750TOBS installation CD, to install the 750TOBS Control program. Normally the sofware installation will start automatically. If this does not happen, run the program “setup.exe” from the CD. The following is a description of the installation procedure:


750TOBS - InstallShield Wizard
Welcome to the InstallShield Wizard for 750TOBS
The InstallShield(R) Wizard will install 750TOBS on your computer. To continue, click Next.
WARNING: This program is protected by copyright law and international treaties.
< Back
Next >
Cancel

Click ”Next”.

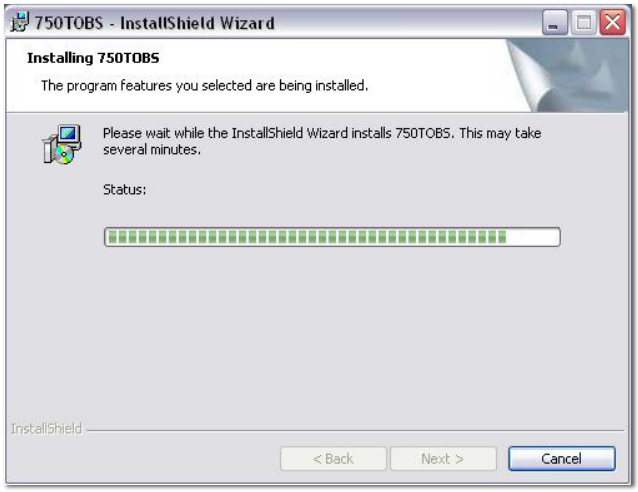
750TOBS - InstallShield Wizard
License Agreement
Please read the following license agreement carefully.
By installing, copying, accessing, or using the SOFTWARE or its OUTPUT you agree to be bound by the terms of this license agreement. If you do not agree to the terms of this license agreement, then DO NOT install or use the SOFTWARE.
THIS SOFTWARE IS NOT FOR SALE. The SOFTWARE is licensed to you as the end user, it is not sold.
Copyright Notice
All content copyright Danish Myo Technology A/S, ©2006, all rights reserved.
I accept the terms in the license agreement
I do not accept the terms in the license agreement
Print
InstallShield
< Back
Next >
Cancel

Accept the terms and click ”Next”.



The program is ready to be installed, click “Install”

The 750TOBS Control program is now installing from the CD.


750TOBS - InstallShield Wizard
Installing 750TOBS
The program features you selected are being installed.
Please wait while the InstallShield Wizard installs 750TOBS. This may take several minutes.
Status:
InstallShield
< Back
Next >
Cancel

Click “Finish”. The control program is now ready to use. Start the program from the icon on the Desktop, or via Start->All Programs->DMT ->750TOBS folder.

750TOBS - InstallShield Wizard
InstallShield Wizard Completed
The InstallShield Wizard has successfully installed 750TOBS. Click Finish to exit the wizard.
< Back
Finish
Cancel

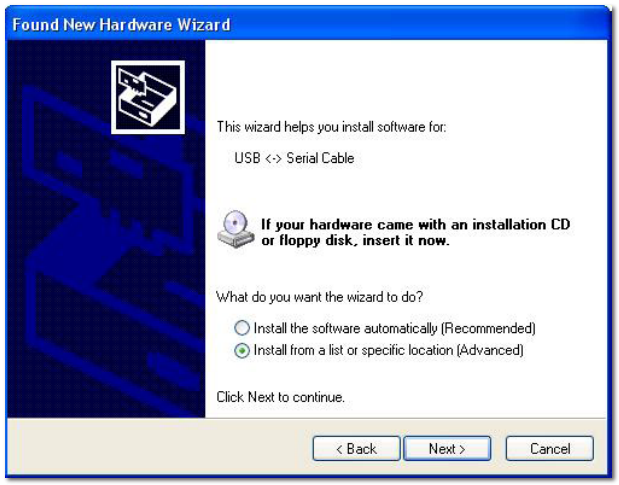
Appendix 6 - VCP – Driver installation

The first time the 750TOBS is connected to the PC, the VCP drivers must be installed. They are on the CD delivered with 750TOBS. To install VCP drivers for 750TOBS under Windows XP follow the instructions below. When the USB cable is plugged into the back of 750TOBS for the first time, this window will open:

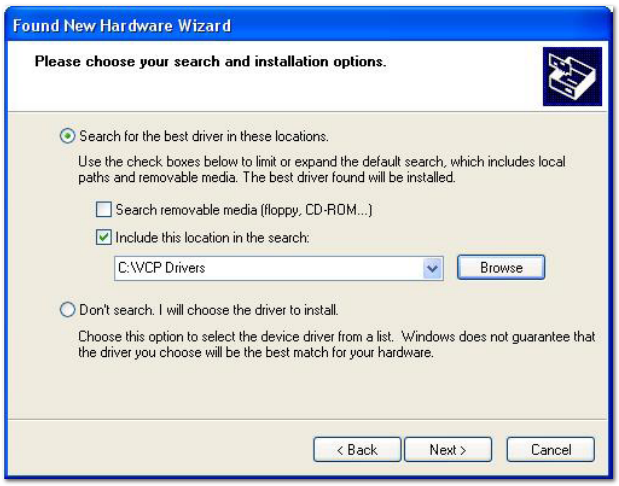
Found New Hardware Wizard
Welcome to the Found New Hardware Wizard
Windows will search for current and updated software by looking on your computer, on the hardware installation CD, or on the Windows Update Web site (with your permission).
Read our privacy policy
Can Windows connect to Windows Update to search for software?
Yes, this time only
Yes, now and every time I connect a device
No, not this time
Click Next to continue.
< Back
Next >
Cancel

Choose “No, not this time”, and click “Next”

Select “Install from a list or specific location (Advanced)” as shown below and then click “Next


Found New Hardware Wizard
This wizard helps you install software for:
USB <-> Serial Cable
If your hardware came with an installation CD or floppy disk, insert it now.
What do you want the wizard to do?
Install the software automatically (Recommended)
Install from a list or specific location (Advanced)
Click Next to continue.
< Back
Next >
Cancel

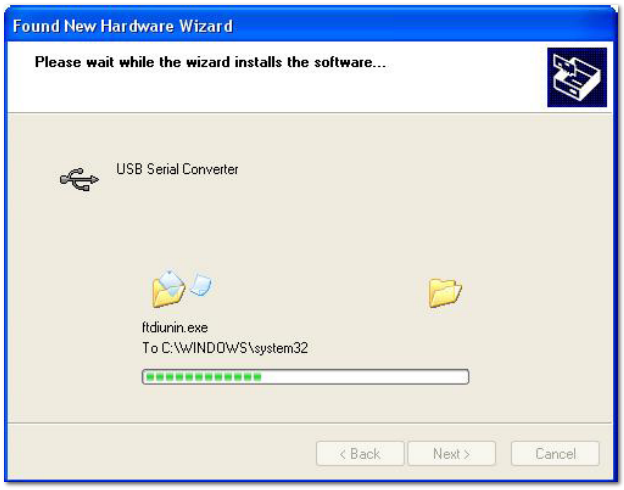
Select “Search for the best driver in these locations” and enter the file path in the combo-box (C:\VCP Drivers” in the example below) or browse to it by clicking the browse button. Once the file path has been entered in the box, click next to proceed.


Found New Hardware Wizard
Please choose your search and installation options.
Search for the best driver in these locations.
Use the check boxes below to limit or expand the default search, which includes local paths and removable media. The best driver found will be installed.
Search removable media (floppy, CD-ROM...)
Include this location in the search:
C:\VCP Drivers
Browse
Don't search. I will choose the driver to install.
Choose this option to select the device driver from a list. Windows does not guarantee that the driver you choose will be the best match for your hardware.
< Back
Next >
Cancel

If Windows XP is configured to warn when unsigned (non-WHQL certified) drivers are about to be installed, the following screen will be displayed. Click on “Continue Anyway” to continue with the installation. If Windows XP is configured to ignore file signature warnings, no message will appear.

Hardware Installation
The software you are installing for this hardware:
USB High Speed Serial Converter
has not passed Windows Logo testing to verify its compatibility with Windows XP. (Tell me why this testing is important.)
Continuing your installation of this software may impair or destabilize the correct operation of your system either immediately or in the future. Microsoft strongly recommends that you stop this installation now and contact the hardware vendor for software that has passed Windows Logo testing.
Continue Anyway
STOP Installation

The following screen will be displayed as Windows XP copies the required driver files.



Windows should then display a message indicating that the installation was successful. Click “Finish” to complete the installation. This has installed the serial converter. The COM port emulation driver must be installed after this has completed.
After clicking “Finish”, the Found New Hardware Wizard will continue by installing the COM port emulation driver. The procedure is the same as that above for installing the serial converter driver.

Now the 750TOBS Controller Software can be opend, and connected to 750TOBS as described in paragraph 3.2.1.

Appendix 7 - Buffer recipes

7.1 Buffer recipes

NOTE: The Schott Duran bottles should not be filled up with more than 800 ml of solution.

NOTE: To minimize the heating time one can benefit by preheating the buffer to 35° C before filling the reservoir bottles.

7.1.2 Physiological Saline Solution (PSS)

1x PSS (1000ml):

Solution 1.

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
NaCl	58.44	118.99	6.954
KCl	74.56	4.69	0.350
$MgSO_4$ - $7H_2O$	246.48	1.17	0.289
KH_2PO_4	136.09	1.18	0.161

Solution 2.

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$CaCl_2$ - $2H_2O$	147.02	2.50	0.368

Solution 3.

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$NaHCO_3$	84.01	25.00	2.100
EDTA	372.24	0.03	0.010
Glucose	198.77	5.50	1.091

1. Dissolve the chemicals in 100 ml double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.

2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 ml.

3. Solution 3 is added to the graduated bottle, which afterwards is filled with additional double distilled H_2O to a final volume of 850 ml.

4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.

5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000 ml. Continue the carbogen bubbling until the pH of the buffer solution reaches 7.4.

25x Concentrated PSS (1000ml)

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
NaCl	58.44	118.99	173.850
KCl	74.56	4.69	8.750
$CaCl_2$ - $2H_2O$	147.02	2.50	9.200

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$MgSO_4$ - $7H_2O$	246.48	1.17	7.225
$KH2PO_4$	136.09	1.18	4.025

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
EDTA	372.24	0.03	0.250

1 Dissolve the chemicals for solution 1 in about 800 ml double distilled H_2O in a 1000 ml graduated bottle. Dissolve the chemicals for solutions 2 and 3 in 75 ml double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.

2. Solution 2 and 3 is added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000.0 ml.

Before use:

3. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.

4. Add

   1.091 g/l Glucose
   2.100 g/l $NaHCO_3$

5. Aerate the solution with carbogen (95%O_2 + 5%CO_2) for at least 20 minutes. If necessary wait further for the pH of the buffer to reach pH 7.4.

7.1.3 KPSS

1 x KPSS (1000ml):

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
KCl	74.56	123.70	9.223
$MgSO_4$ - $7H_2O$	246.48	1.17	0.289
KH_2PO_4	136.09	1.18	0.161

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$CaCl_2$ - $2H_2O$	147.02	2.50	0.368

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$NaHCO_3$	84.01	25.00	2.100
EDTA	372.24	0.03	0.010
Glucose	198.77	5.50	1.091

1. Dissolve the chemicals in approximately 100 ml double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.

2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 ml.

3. Solution 3 is added to the graduated bottle that is filled with additional double distilled H_2O to a final volume of about 850 ml.

4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.

5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000.0 ml. Continue the carbogen bubbling until the pH of the buffer solution reaches 7.4.

25 x KPSS (1000ml)

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
KCl	74.56	123.70	230.575
$CaCl_2$ - $2H_2O$	147.02	2.50	9.200

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
$MgSO_4$ - $7H_2O$	246.48	1.17	7.225
KH_2PO_4	136.09	1.18	4.025

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/l)**	**Conc. (g/l)**
EDTA	372.24	0.03	0.250

1. Dissolve the chemicals for solution 1 in about 800ml double distilled H_2O in a 1000 ml graduated. Dissolve the chemicals for solutions 2 and

2. 3 in 75 ml double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.

3. Solutions 2 and 3 are added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000 ml.

Before use:

4. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.

5. Add

   1.091 g/l Glucose
   2.100 g/l $NaHCO_3$

6. aerate the solution with carbogen (95% O_2 + 5% CO_2) for at least 20 minutes. If necessary wait for the pH of the buffer to reach pH 7.4.

Appendix 8 - 750TOBS controller

Appendix 8 is the 750TOBS Software manual without the Data acquisition. This software is used if the user prefers a different Data acquisition program.

8.1 The 750TOBS-Software User Interface

The first time the DMT Tissue Organ Bath is turned on, the VCP drivers must be installed as described in Appendix 6.

8.1.1 Connect to 750TOBS:

Setup->Connect to 750TOBS:
The connection to the 750TOBS is automatic. When the connection is performed, the message “Connected: COMx” is displayed in the status bar in the bottom left corner of the program window. If the connection fails, the message display is: “Not connected”.
In case the connection fails try connecting by clicking on‘Setup->Connect’. If the connection fails again, check if the USB cable is mounted correctly and 750TOBS is turned on.

If two 750TOBS are connected to one PC use both programs. **Always connect 750TOBS-Software A before 750TOBS-Software B.**

8.2 Control windows

8.2.1 The Main window

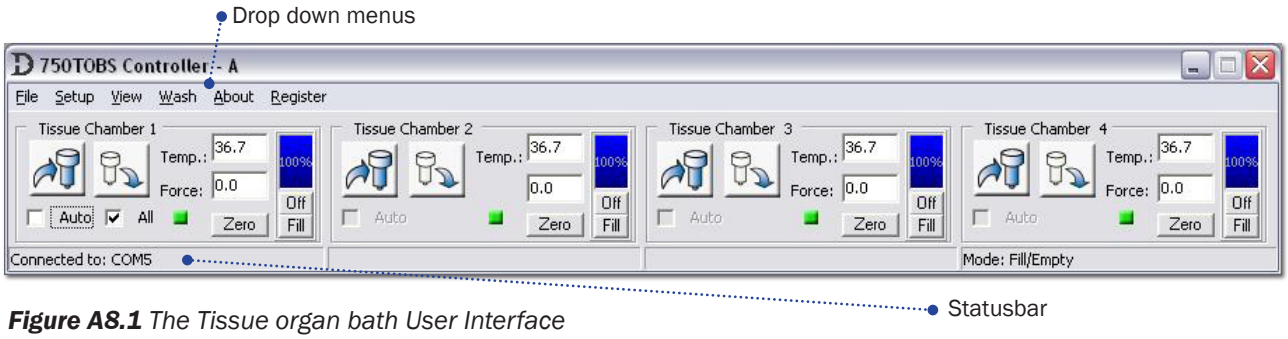
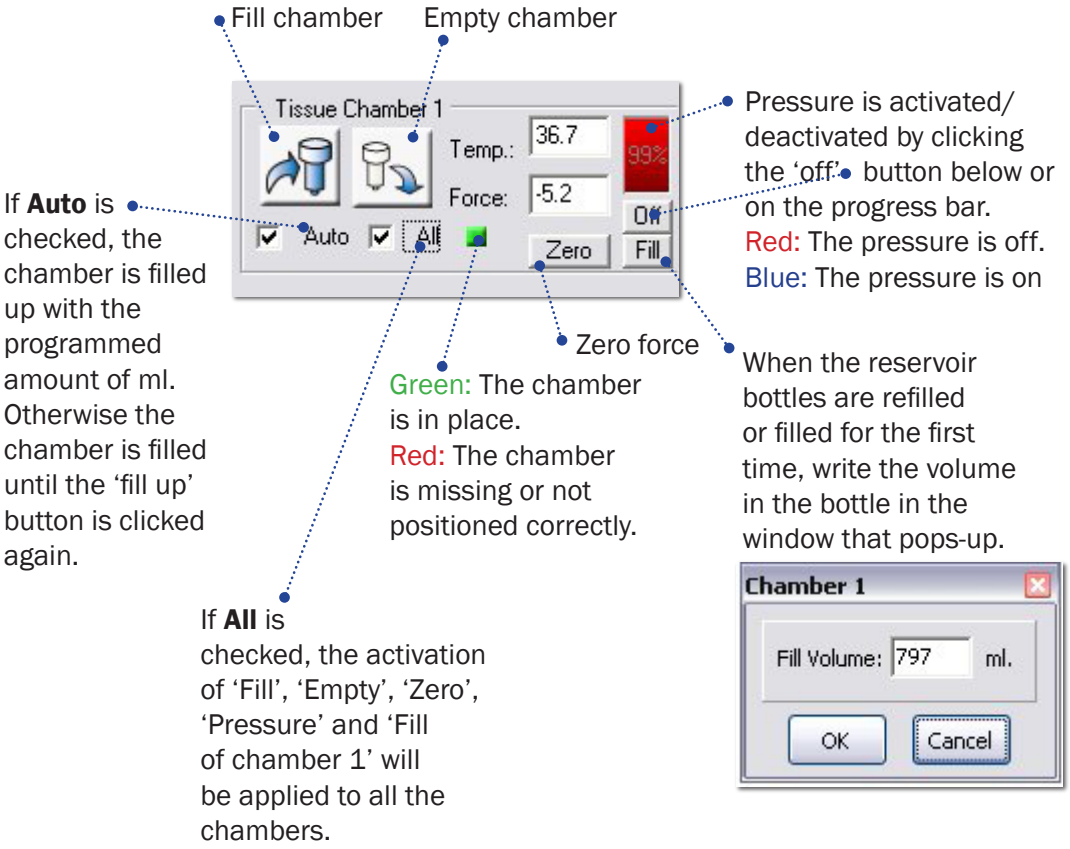


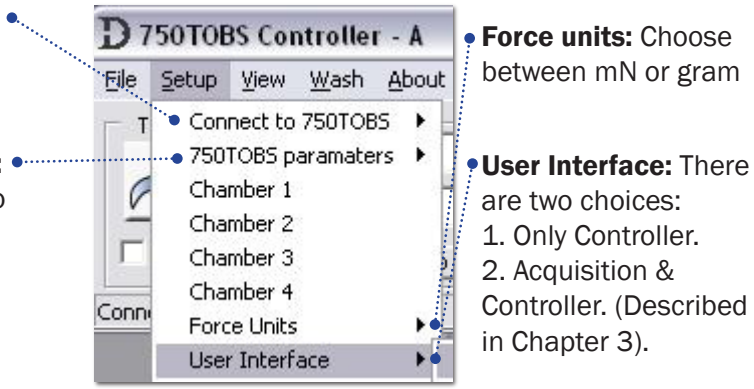
Figure A8.1 *The Tissue organ bath User Interface*

Tissue chambers controls


Fill chamber
Empty chamber
Tissue Chamber 1
Temp.:
36.7
Force:
-5.2
Auto
All
Zero
Off
Fill
Pressure is activated/ deactivated by clicking the 'off' button below or on the progress bar.
Red: The pressure is off.
Blue: The pressure is on
If **Auto** is checked, the chamber is filled up with the programmed amount of ml. Otherwise the chamber is filled until the 'fill up' button is clicked again.
Zero force
Green: The chamber is in place.
Red: The chamber is missing or not positioned correctly.
When the reservoir bottles are refilled or filled for the first time, write the volume in the bottle in the window that pops-up.
Chamber 1
Fill Volume:
797
ml.
OK
Cancel
If **All** is checked, the activation of 'Fill', 'Empty', 'Zero', 'Pressure' and 'Fill of chamber 1' will be applied to all the chambers.

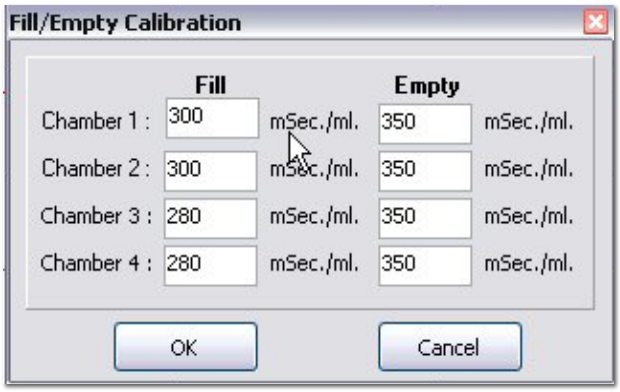
8.2.2 Drop down menus

8.2.2.1 Setup menu



8.2.2.2 Fill/Empty calibration

Setup->750TOBS parameters ->Fill/Empty calibration



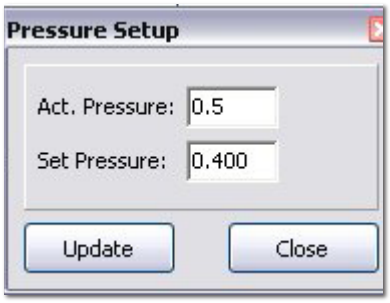
The amount of fluid filling the tissue chamber is time regulated. Calibration of each chamber is performed with the marked tissue chamber provided with the calibration kit (20 ml).

Adjust the numbers of each tissue chamber until the amount of fluid is correct, the numbers indicates msec/ml.
If the amount of fluid is to small, the number (msec/ml) must be higher. If the amount of fluid is to high the number (msec/ml) must be lower.

8.2.2.3 Pressure

Setup->750TOBS parameters->Pressure

Adjustment and on-line reading of pressure. This setting is normally **not** changed.



Actual pressure

'Act. Pressure' provides an on-line reading of the actual pressure in the reservoir bottles.

Set pressure

To set or reset the target pressure
(0.01- 0.999 Bar).
(Default: 0.400)

Update

'Update' provides an on-line reading of the actual pressure.

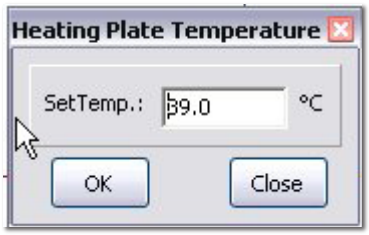
NOTE: When the pressure is reset press the small button that appears to the right of the Set Pressure Input field.
If the pressure is changed, the flow must be calibrated again, as described in paragraph 3.2.3.10

8.2.2.4 Heating plate temperature

Setup->750TOBS parameters->Heating plate temperature

Adjusts the temperature on the heating plate, on which the reservoir bottles are placed. The temperature here is chosen to be higher than the bath temperature to avoid gas formation in the lines and to correct for cooling during refilling of the chambers.

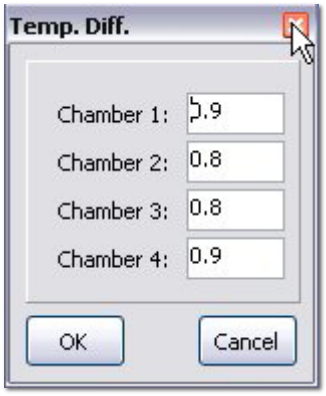
'Set Temperature' is used to set the set temperature of the heating plate for the reservoir bottles. (Default: 39°C).
The Set Temp. is updated when clicking the OK button.
NOTE: To minimize the heating time one can benefit by pre-heating the buffer to 35°C before filling the reservoir bottles for the first time in the day.



8.2.2.5 Temperature difference

Setup ->750TOBS parameters->Temperature difference

Adjustment between the actual temperature and target temperature.



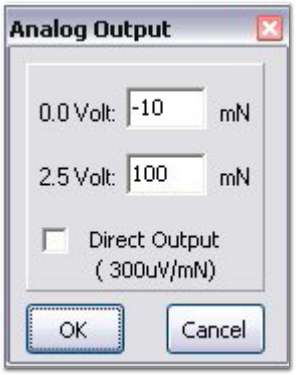
The Set temperature and Actual temperature may not be equal due to the ambient temperature in the lab. It is possible to compensate this with this setting.

Example: If the *Set.Temp* for Chamber 1 is 37.0 °C and the actual temperature measured in Chamber 1 is 36.2 °C . The *Difference setting* for Chamber 1 must be 0.8.

8.2.2.6 Analog output

Setup ->750TOBS parameters-> Analog output

The output is from 0.0 to 2.5 Volt. (Resolution is 0.61 mV). The span in this window can be adjusted.
In the example above the range is set to 110 mN . The output is 0.0 Volt, if the force is -10mN and 2.5 Volt if the force is 100mN.



The internal sample frequency is 20Hz on each channel. Any digital filtering in your data acquisition software, must be set above 20Hz.

If the setting *Direct output* is choosen the settings above is not used. The output is then routed directly from the transducer :

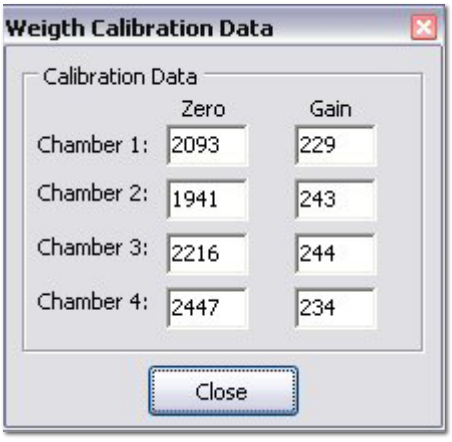
Offset: 2.5 Volt
Output: +/-300 µV/mN

If *Direct output* is used the signal is not filtered and the output is only limited by the transducers response time, it is approximately 10 mS.

8.2.2.7 Weight calibration data

Setup ->750TOBS parameters->Weight calibration data

A read out of the *Force calibration data.* (Only for internal use, if there are some problems with a transducer).



8.2.2.8 Wash

Setup ->750TOBS parameters ->Wash

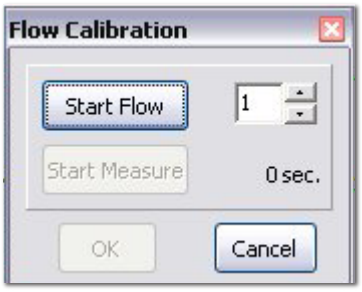
Programming of the wash sequence.
The wash sequence works by overflow.
In the example to the right the wash sequence is set to:
Wash 4 sec. every 1 min. 2 times, then wash 3 sec. every 1 min. 5 times.
If 'next wash' is not checked, there will only be one wash

8.2.2.9 Flow Calibration

Setup ->750TOBS parameters->Flow calibration

Before this calibration is performed, the low flow shunt supplied in the calibration kit must be mounted on the tubing inside the reservoir bottle. Calibration of flow in Constant Wash mode is performed by the marked Tissue chamber provided with the calibration kit (5 ml). On the Tissue chamber there are two marks used for the calibration. The calibration is done one chamber at the time.

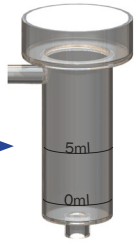
1. Turn off oxygenation.

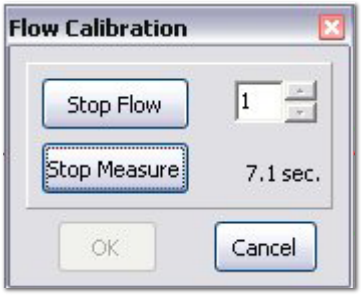


2. Choose the chamber to calibrate.
   Click Start flow.

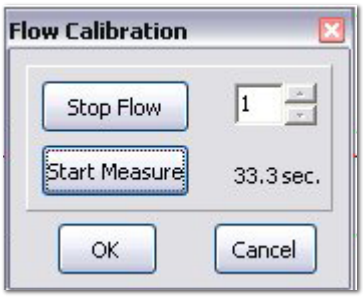
3. When the surface of the fluid reaches the bottom mark on the calibration tissue chamber, click the Start Measure button.Check this by looking at the line at eye level and look for the bottom of the fluid meniscus to reach the line!

4. When the surface of the fluid reaches the top mark on the calibration tissue chamber, click the Stop Measure button.





5. Click the OK button. Flow calibration for the chosen chamber is now finished.



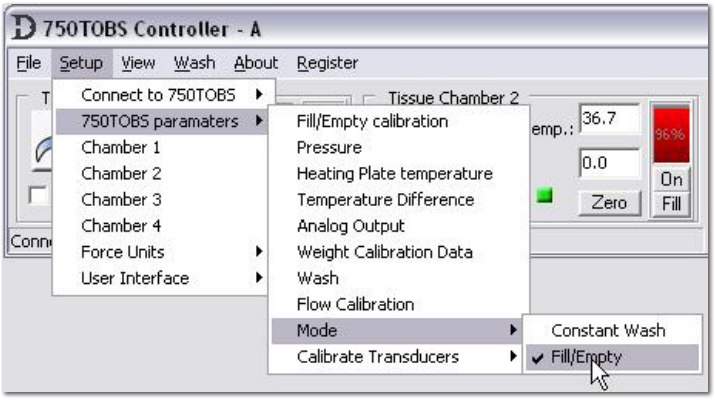
Repeat step 1-4 for all the chambers.

8.2.2.10 Mode select

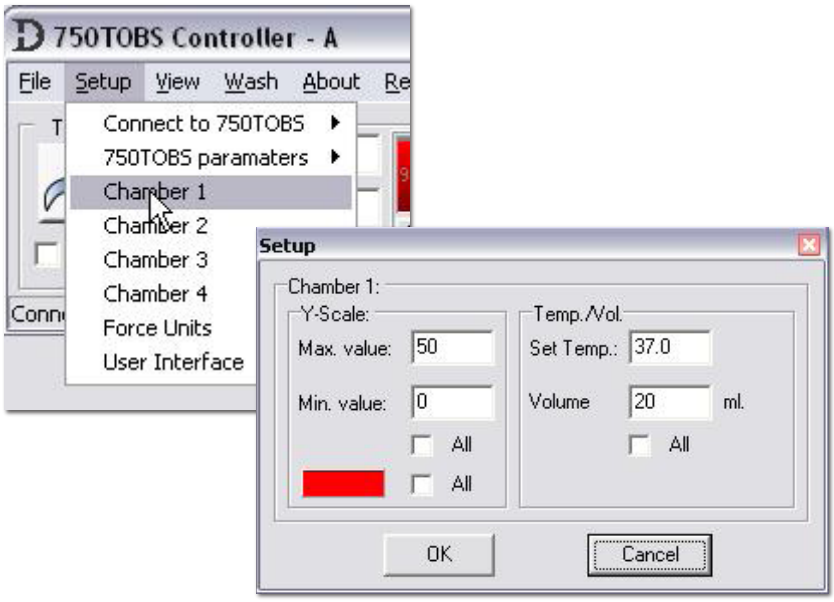
Setup ->750TOBS parameters-> mode ->

The 750TOBS has two different wash modes. Constant wash and Fill/empty.

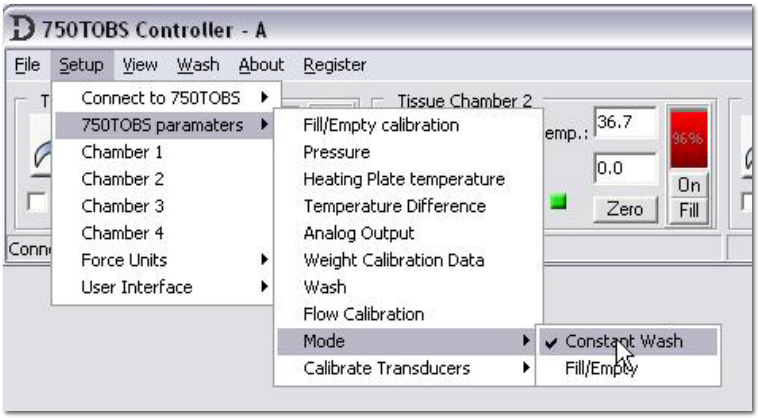
The classic way of changing solution in an organ bath system is to drain the entire bath and refilling it with fresh buffer via a Fill/empty routine. The advantage of this method is that the concentration of drug declining fast especially if this is repeated 3 times or more. The 750TOBS can do this automatically via the wash menu and the buffer comes from the pre-heated buffer reservoir in the system. Therefore compared to the classic approach, washing is easy, convenient and fast. A disadvantage of this fill/empty method is that the tissue is briefly suspended in air, without nutrients and stimulation, when used, needs to be suspended.



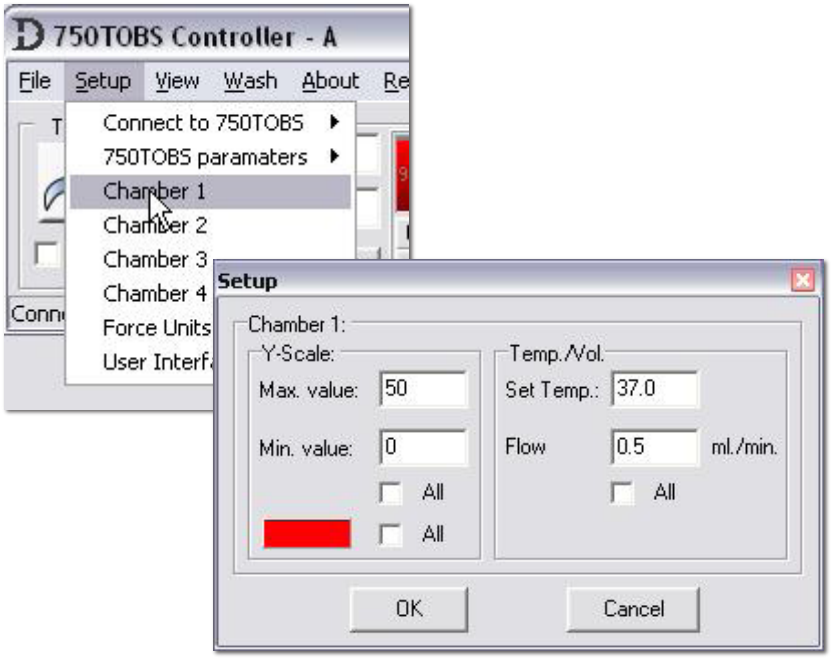
When the fill/empty mode is selected go to **Setup->Chamber 1-4** to adjust the settings.



A second new method in the 750TOBS is to perform a constant wash at a preset flow rate. In the 750TOBS the fresh warm buffer from the reservoir is filled from below and excess buffer is evacuated from the overflow on the top. The flow rate can be programmed from 0.5 - 5.0 ml/min . Although it may take a little more time to completely wash any drugs away, the advantages are that the tissue is never in air, stays in warm buffer and stimulation, if used, does not need to be interrupted. The user is encouraged to try both methods and based on own judgment determine what is best for the experimental conditions.



When the Constant wash mode is selected go to **Setup->Chamber 1-4** to adjust the settings.



Important: When the mode is changed, the shunt inside the bottle must be changed too.

The Constant wash mode requires the **Constant flow shunt** and Fill/Empty mode requires the **Fill/Empty flow shunt**.

Important: Make sure that the pressure is OFF before changing the shunts.

A warning noting that the pressure is turned off, is shown when the mode is changed.

Attention !
You are changing mode.
Please check that the right shunt is mounted in the bottle.
Please note that the pressure is removed.
Close

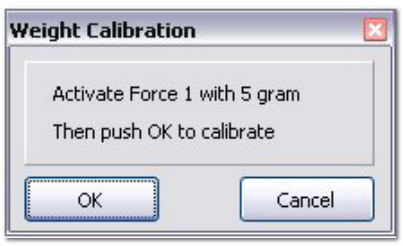
Constant flow shunt
(Hypodermic needle 0,4 x 40mm)

Fill/Empty flow shunt

Accessories, please contact DMT for purchase.

8.2.2.11 Weight calibration

By activating **Setup ->750TOBS parameters-> Calibrate transducers -> Chamber X** a message appears as shown to the right.
Place the 5g weight on the transducer. Wait until the relative force reading is stable. Click OK.
The force applied on the force transducer should mimic the stretch created by the contraction of a mounted ring preparation.



8.3.1 View

Hide/show the controller:
Click View->Slide Up to hide the controller.
Click View->Slide Down to show it again

8.4.1 Wash

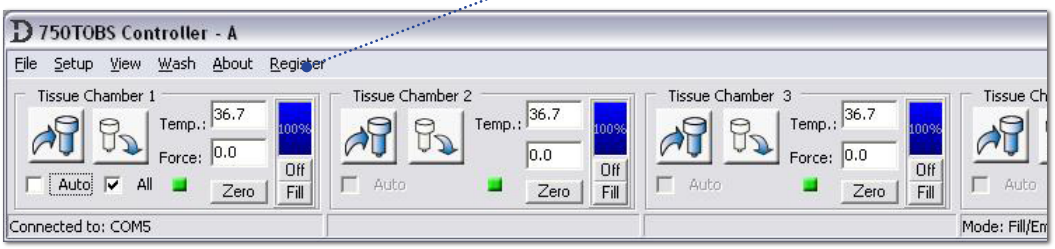
Start washing

8.4.2 About

In the About box, software, firmware and hardware ID, for the 750TOBS is shown. There are also links to DMT support and website.

8.4.3 Register

If you want to register the software, click Register and your Web browser will open. It is now possible to register at DMT´s website.



Appendix 1 - Terms of warranty

Warranty

DMT A/S warrants to the original user that myograph systems manufactured by DMT A/S will be free from defects in materials and workmanship for a period of three years after the date of delivery. DMT A/S will repair or replace any defective part, subject to the conditions, limitations and exclusions.

Exclusions

Force and pressure transducers, separately or part of myograph systems manufactured by DMT A/S, are disclaimed from any warranty.

Limitations

This warranty shall not apply to equipment subjected to accidental damage, improper use, alteration, or deterioration.

Warranty on third-party products will be as determined by their respective manufacturer.

DMT A/S shall not be liable for consequential, incidental, special, or other direct or indirect damages resulting from economic loss or property damage sustained by you or any end user from the use of the products sold or services rendered hereunder.

Warranty returns

A “Return Material Authorisation” (RMA) number is required for all returns. This number should be clearly indicated on all returned myograph systems.

Products damaged due to improper or inadequate packaging when returned for RMA purposes are not granted warranty coverage.

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